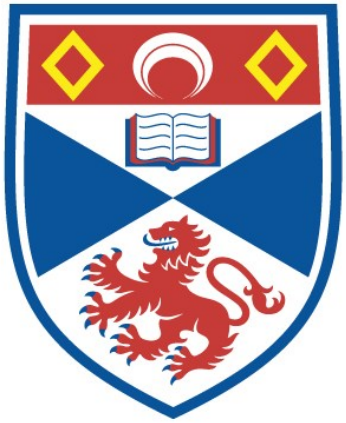
# STUDIES ON ABSORPTION FROM MAMMALIAN INTESTINE

**Helen Ogilvie Wood**

**A Thesis Submitted for the Degree of PhD**
**at the**
**University of St Andrews**



**1945**

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A THESIS

PRESENTED FOR THE DEGREE

OF

DOCTOR OF PHILOSOPHY

OF

THE UNIVERSITY OF ST. ANDREWS

BY

HELEN OGILVIE WOOD, B.Sc.

## Certificate

I certify that Helen Ogilvie Wood, B.Sc. has spent nine terms on research work under my direction and that she has fulfilled the conditions of Ordinance No.16 (St. Andrews) so that she is qualified to submit the following Thesis in application for the degree of Ph.D.

Professor of Physiology,

University College,

Dundee.

## Declaration.

I hereby declare that the following Thesis is a record of results of experiments carried out by me and that the Thesis is my own composition and it has not been previously presented for a higher degree.

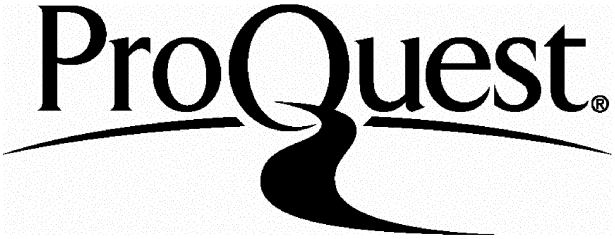
The research work was carried out in the Physiological Laboratories of University College, Dundee under the direction of Professor R.C. Garry, D.Sc.

## University and Research Training.

I entered University College, Dundee in October, 1937 and graduated in October, 1941 with Second Class Honours in Physiology.

In October, 1941 under the direction of Professor R.C. Garry, D.Sc. I commenced the research work which forms the subject of this Thesis. In October, 1941 I was appointed temporary assistant to Professor Garry and have acted in that capacity during the sessions 1941-42, 1942-43, 1943-44 and during the present session.

# STUDIES ON ABSORPTION FROM MAMMALIAN INTESTINE.



"Students of absorption ----------have in the past relied too much on the classical methods of biochemistry. Insufficient attention is paid to the cell as an active organ".

Danielli and Davson (1943)

Permeability of Natural Membranes. Cambridge University Press.

V. Summary.

# GENERAL INTRODUCTION

## GENERAL INTRODUCTION.

In the historical development of the conceptions of the mechanism of absorption from the intestine it is evident that, from the very beginning, the views fall into two main categories, namely:-

(1) that absorption from the intestine is purely a physico - mechanical process.

(2) that the process of intestinal absorption is due to the physiological activity of the living intestinal cells and that the phenomena observed cannot be explained on known physical laws.

Amongst the earliest recorded views on the process of absorption were those of Rudolphi (1800) and Magendie (1825) who believed that absorption was caused by mere physical "imbibition" while, on the other hand, Tiedemaner and Gmelin (1820) believed that the villi acted like inverted secreting glands. With the advance of knowledge concerning osmotic phenomena, Dutrochet (1826) put forward his theory of endosmosis to explain the process of intestinal absorption. For many years an osmotic explanation in a modified form held the field.

However, in 1851, Brücke put forward again the theory propounded by Lieberkühn in the previous century that the peristaltic pressure brings about absorption by forcible filtration of the gut contents into the living tissues. Later, Voit and Bauer (1869) supported this view as a result of their investigations on the absorption of protein solutions, serum and salts. They concluded that osmosis was not the cause of intestinal absorption but that intra - intestinal pressure was the primary factor involved. These deductions were

based partly upon the erroneous idea that the passage of solutions through the intestinal wall should imitate the diffusion of the same solutions through artificial membranes. The fact that animal membranes may vary in their permeability was not known at the time.

Hoppe - Seyler (1881) was the first to cast grave doubts on the adequacy of a mechanistic theory of osmosis and filtration to explain all the phenomena observed during intestinal absorption. He stated definitely that absorption from the intestine is a function of living epithelial cells. The evidence was based upon the action of the gut in cholera and when poisoned by certain toxic substances. Since, in these cases, normal absorption did not take place Hoppe - Seyler concluded that the vital activity of the epithelial cells had been destroyed. In cholera, where the epithelium of the intestine is largely shed, the absorption is at a standstill although thinning of the membrane might favour osmotic transfer of the gut contents. Waymouth Reid (1900) pointed out that these experiments did not necessarily prove that the epithelium actively transferred the solutions into the blood in the normal state of affairs. He considered that the epithelium might act as "a barrier, physically impermeable to certain substances in solution in the plasma and exerting osmotic pressure and that with the removal of the barrier such substances can diffuse over into the gut so that the value of their osmotic pressure as a factor in absorption of water is annulled".

Heidenhain (1894) and his pupils Leubusches (1885), Gumilewski (1886) and Röhmann (1887) came to the same conclusion as that of Hoppe - Seyler but with one important modification. Heidenhain believed that a certain portion of soluent and solute was absorbed by a process of osmosis while another portion was absorbed by the physiological activity of the living cell and did not obey the known physical laws. By the "activity of the living cell" Heidenhain maintained that the cell could exert an influence upon its physical or chemical processes.

Cohnheim (1898-1900) made the discovery that the absorptive process was a one - way mechanism from the gut lumen. He showed that the diffusible blood constituents did not, under normal conditions, pass into the intestine. There were two main factors in Cohnheim's theory of absorption -

(1) He postulated an impermeability to body fluids due to the activity of the capillary endothelium which brought about the osmotic equilibrium between intestinal solutions and the blood without the passage of blood constituents into the gut solutions.

(2) He also postulated that the gut wall was able to take up the contents of its lumen through the intermediation of the vital activity of the epithelial cells and that this process was free of physical influences. Cohnheim rejected the idea put forward by Heidenhain that the cellular activity of the epitheluim might be affected by the osmotic pressure of the intestinal contents.

Waymouth Reid (1892-1902) on the same general grounds as Heidenhain and Cohnheim, claimed that intestinal absorption was due to the

intervention of the epithelial cells. He stated, however, that the forms of energy utilised were not other than those known to the physicists. Reid attributed the power of absorption solely to the activity of the epithelial cells and consequently his theory of intestinal absorption was in closer agreement with Cohnheim than with Heidenhain who postulated that osmotic pressure partially influenced the process of absorption. Reid based his argument upon a series of experiments in which he showed serum could be absorbed from the intestine under conditions in which filtration, osmosis and adsorption were said to be excluded. Injury to or removal of the epithelial cells of the intestine resulted in a depression of the absorption mechanism. Reid argued that such procedures should accelerate the absorptive process since they favoured the purely physical forces of adsorption, osmosis and filtration. In fact, any condition depressing cellular activity tended to reduce the absorptive action. By means of a simple diffusion apparatus called an osmometer, Reid, using fluids of the same composition on either side of the membrane showed that the movement of substances took place across an isolated surviving piece of intestine. The movement took place from the fluid in contact with the mucosa to that bathing the opposite serosal side of the intestine. By using fluids of the same composition on both sides of the membrane he excluded the influence of osmotic pressure. In this way, it was argued, the active force must be a "vital" function inherent in the epithelial cells.

The work of these three authors provided the basis for the theory that the physiological activity of the intestinal cells was responsible

for absorption from the intestine.

Hamburger (1896-1908) did not accept the theory that the process of absorption was due to "vital" cell activity but from the results of a series of experiments designed to determine the effect of intra-intestinal pressure upon absorption came to the conclusion that, although physiological and pathological changes could affect the physical forces involved, they did not cease to be purely mechanical. He claimed to have imitated all the phenomena exhibited in the intestinal absorption of sodium chloride and serum with artificially constructed membranes.

Höber (1898-1914), comparing the rates of diffusion of various solutions of salts with their speed of absorption in the intestine, concluded that although physical factors did play a part in the mechanism of absorption they did not explain the whole process.

Wallace and Cushny (1898-1899) compared the rates of absorption of a large number of equimolecular salt solutions with a one per cent solution of sodium chloride and found that the rates of absorption did not correspond with the diffusion rates. Salts which were easily dissociated into ions of high speed were not always absorbed at a faster rate than salts less easily dissociated and whose ions possessed a lower speed.

It was the careful study of the absorption of the monosaccharides which led eventually to the solution of the problem which has divided the mechanists and vitalists. Cori (1925) finally established the fact that, although the known physical laws are factors of great importance in the process of absorption, they cannot

explain all the phenomena concerned with absorption. Cori used unanaesthetised rats as his experimental animals. He determined the rates of absorption of monosaccharides from the small intestine by feeding a known amount of a given sugar in solution through a stomach tube, killing the animal and estimating the amount of sugar remaining in the intestine. He was able to determine with great accuracy the amount of sugar absorbed in a given time by a rat of known weight. Within fairly wide limits, the amount of glucose absorbed did not vary with the concentration of the solute. In other words, the process of absorption did not conform to the laws of diffusion and osmosis. If absorption was merely a matter of diffusion the number of ions bombarding the absorbing surface in unit time would obviously be a factor of great importance.

In 1930, Auchinachie, McLeod and Magee studied the diffusion of solutes through the surviving isolated intestine of rabbits. These workers found that 0.2 per cent potassium iodide solution passed more rapidly through dead gut than living. Blood-isotonic glucose (5.4 per cent) also diffused more rapidly through dead gut. In both these cases the gut was killed with hot isotonic saline at 65°C for four minutes. All methods of killing increased the permeability of the gut to glucose and dihydroxyacetone. $\frac{M}{2}$ xylose solution diffused more rapidly than $\frac{M}{2}$ glucose solution through dead rabbit gut but sometimes in "living" gut, glucose passed out more rapidly than xylose. Lowering of the temperature allowed glucose and xylose to diffuse out at the same rate from the intestine of the cat and the rabbit. The criticism which has been levelled

at this work will be discussed later.

Cori's method of determining the rate of absorption of monosaccharides suffered from one defect: namely the emptying time of the stomach was variable. To overcome this, Verzár injected the sugar solutions directly into loops of small intestine of anaesthetised rats. The amount of sugar remaining in the loops, after a given period of time had elapsed was estimated. There is a remarkable agreement between Verzár's and Cori's results. Both workers found that hexoses such as glucose and galactose were absorbed at a much faster rate from the small intestine of the rat such as xylose and arabinose.

In 1930, Lundsgaard described an experiment in which it was shown that frog's muscle poisoned with monoiodoacetic acid inhibits the formation of lactic acid in striped muscle. He also found that yeast fermentation which starts with a carbohydrate - phosphate combination is inhibited by monoiodoacetic acid. Yamasaki (1930) found that this fermentation will continue if hexosephosphate is taken instead of sugar. It was concluded, therefore, that monoiodoacetic acid stopped the phosphorylation of sugar. Later work showed that monoiodoacetic acid influences phosphorylation processes indirectly. Moniodoacetic acid is believed to inhibit the oxydoreductions leading from hexosephosphoric acid via triosephosphoric acid to phosphoglyceric acid and glycerophosphoric acid. Since these are part of the processes connected with hexosephosphoric acid production this will also be inhibited.

Verzár and his co-workers postulated similar phosphorylation syatems in the mucous membrane of the small intestine to account for the rapid

selective absorption of glucose. The first experiments were carried out by Wilbrandt and Laszt (1933). Luundsgaard (1930-31) had found that '/5000 monoiodoacetic acid inhibits yeast fermentation and in the first series of experiments the intestine was poisoned locally by adding the required dose of iodoacetic acid to the blood - isotonic glucose or xylose injected into ligated loops of intestine in the rat. In normal animals 72.8 per cent glucose was absorbed; in the poisoned animals only 54.8 per cent glucose was absorbed. The amount of xylose absorbed in normal animals was 21.4 per cent and in poisoned animals this value was 25.4 per cent - an increase of 20 per cent of the amount absorbed. The ratio $\frac{\text{percentage glucose absorbed}}{\text{percentage xylose absorbed}}$ in one hour was 3.4: 1 in the normal rat and 2.2: 1 in the poisoned rat.

In later experiments the poison was injected subcutaneously "thus avoiding any direct damage to the epithelium of the mucosa." The dose given was 0.12 - 0.16 mgms per gm. body weight giving a concentration in the body of 1/6000 to 1/8,000. This caused a decrease of glucose absorption from 73.3 per cent to 24.3 per cent. The relationship between absorbed glucose and absorbed xylose was again 3.4: 1 in normal rats but 1.1: 1 in poisoned animals. The action of monoiodoacetic acid on the absorption of galactose, fructose, arabinose and rhamnose was also studied. The rate of absorption of galactose and fructose was lower in iodoacetate-poisoned rats than in normal rats. The rate of absorption of galactose in the poisoned rats was reduced to only half the rate of absorption in normal rats. The rate of absorption of arabinose and rhamnose was slightly higher in iodoacetate - poisoned rats than in normal rats. In the case of

rhamnose 21.1 per cent was absorbed by a normal rat in one hour, 28.5 per cent was absorbed by iodoacetate - poisoned rat.

From these experiments, where iodoacetic was injected subcutaneously, Verzár and his co-workers concluded that monoiodoacetic acid inhibits the transformation of glucose inside the mucosa and therefore inhibits that part of its absorption which is due to a special synthetic process, namely phosphonylation. Pentoses were said not to be phosphorylated during absorption since monoiodoacetic acid did not decrease their rate of absorption in rats. Verzár claimed that phosphorylation of glucose and galactose during absorption caused a steeper diffusion gradient for these sugars than for sugars which are not phosphonylated e.g. pentoses. Monoiodoacetic acid inhibited the synthetic process concerned with the selective absorption of glucose and galactose and so abolished the higher diffusion gradient with the result that glucose and galactose ought to have been absorbed at the same rate as xylose and and rhamnose. This holds for glucose but galactose is absorbed at higher rate than xylose in isdoacetate poisoned rats.

Venyár and his co-workers supported their evidence for a phosphonylation mechanism in the small intestine by the following work.

(1) Using phosphate buffer solutions, Laszt (1935) tested the influence of pH upon the absorption rates of glucose and xylose. He found that the absorption rate of glucose was greatest at pH 7.0. The absorption rate of xylose was unaffected by pH. Similar results were obtained using acetate and borate buffers. Laszt concluded

that the selective absorption of glucose was brought about by a process involving chemical reactions which had an optimum pH of 7. The absorption of xylose, being uninfluenced by changes in pH, was said to be a physico - mechanical process involving simple diffusion across a membrane.

(2) Using mucosal extracts in vitro Wildbrandt and Lasyt showed that the mucosal extract phosphorylated blood-isotonic solutions of glucose. The evidence for this was the fact that there was the fact that there was a reduction in the amount of inorganic phosphate Addition of 1/5000 iodoacetatic acid prevented the disappearance of inorganic phosphates. The mucosal extract was said not to phosphorylate mannose or xylose. $\frac{N}{100}$ Soduim Fluoride was reported to have no inhibiting influence on the extract.

(3) The organic phosphate content of the intestinal mucose increased during the absorption of glucose but remained unaltered during the absorption of xylose. Rats were fed with different sugars, the mucosa scraped off and extracted with trichloracetic acid. This finding was confirmed by Lundsgaard (1939) in rats but was not found to occur in cats.

(4) Verzar tried the effect of other poisons on the selective absorption of glucose. Sodium cyanide injected subcutaneously in doses of 0.8 mgms per 100 gms body weight rat. The animals survived one day. Higher doses were not given because the intestinal contents in such animals contained blood. Results were erratic. In two cases out of four, the absorption of glucose was normal, in two others both the absorption of glucose and xylose were diminished.

Althausen, Anderson and Stockholm, 1939; Clark and Mackay, 1942). Marazzi (1940) has reported that fasted normal rats, sham operated and unilaterally adrenalectomised rats showed a decreased rate of absorption of the same order as that found in adrenalectomised rats.

Criticism of Verzár's phosphorylation theory came mainly from Westenbrink and from Klinghoffer. Westenbrink (1936) injected 0.12 mgms monoiodoacetic acid subcutaneously into rats. Using Cori's method of determining the rate of absorption of carbohydrates, he found that the rate for glucose was lowered. When absorption started half an hour after the injection of monoiodoacetate the xylose absorption rate was not affected but it was lowered when an hours interval was allowed. By Verzár's method, using urethane and iodoacetate together, xylose absorption was greatly slowed. Westenbrink concluded that the action of monoiodoacetic acid was in part at least due to its action upon the circulation. Westenbrink also showed that the pigeon was more sensitive to iodoacetic acid than the rat. Doses which did not kill the pigeon in two hours did not affect glucose absorption.

Klinghoffer (1938) also found that the rates of absorption of xylose and of soduim chloride, as well as that of glucose, were lowered when monoiodoacetic acid was injected subcutaneously. The rate of glucose absorption did not fall to quite the normal xylose level. The dose of monoiodoacetic acid injected subcutaneously varied from 0.6 - 0.2 mgms per gm. rat. The pathological findings were not consistent, gastro-intestinal lesions being the most consistent. Klinghoffer decided that, as there was no evidence that

the absorption of xylose and sodium chloride was in any way connected with phosphorylation processes, and as the intestinal pathological changes were so severe, inhibition of glucose absorption by iodoacetate could not be attributed to a specific effect on phosphorylation processes. Klinghoffer also stated that the defect in glucose absorption in rats following administration of sodium iodoacetate could not be attributed to adrenal damage as lesions of the adrenal glands could not always be found in iodoacetate poisoned animals.

More recently Danielli (1943) suggested that poisons inhibiting phosphorylation may prevent absorption not because the substance to be absorbed is phosphorylated but due to the deprivation of energy derived from the metabolism of carbohydrate for some other process actually concerned in the absorption. He finally concludes that "the intestine is such a complex tissue and so complicated by active processes that it does not seem profitable to make further comment upon it at the present time".

The whole problem of absorption from the intestine is obviously very complex. There is obviously no a priori reasons why the mechanism of absorption should be the same for different substances or even the same in different species. It is clear that our knowledge is still very defective, work for many years by many investigators will certainly be required before we can hope to begin to solve the problems of absorption. In this thesis, an attempt is made to do no more than investigate as critically as possible certain of the outstanding problems accessible to modern methods of approach.

# SECTION A.

## Surface Area of the Small Intestine in the Rat and Cat.

Introduction.

Method.

Results.

a) Histological Appearances.

b) Area of the Mucous Membrane.

Discussion.

a) Absolute Values.

b) Relative Values.

Summary.

## Surface Area of the Intestinal Mucosa in the Rat and in the Cat.

### I. Introduction.

Within recent times there has been a renewal of interest in the problems of absorption from the intestinal tract. It is more than probable that one factor of prime importance is the area of the mucous membrane across which absorption takes place. Unfortunately this fundamental aspect seems to have received but scant attention. Admittedly, attempts have been made to estimate - measure implies probably too great a degree of accuracy - the intestinal mucosal area in various animals, but the older data and methods of presentation leave much to be desired.

It seemed worth while, therefore to try to obtain figures for the intestinal absorbing surfaces both in the rat and in the cat, the animals most frequently used in absorption studies. Moreover, since in recent times work has been done on the relative rates of absorption in the cranial and caudal regions of the small gut, an attempt was made to compare the area of the mucous membrane in the cranial region of the jejunum with that in the caudal region of the ileum.

Warren (1939), with improved technique, made a detailed study of the mucosal area at different levels in the intestine of one dog and his summary of the information available at the present time is given below. There seem to be, however, no data for the rat or for the cat, two animals, as mentioned above, most frequently used for experiments on absorption.

TABLE I.

| Author. | Animal | Condition of Tissue | Intestinal Level | Measurements. |
|---|---|---|---|---|
| Heidenhain (1888) from Spee (1885) | Dog | Fixed specimens (osmic acid) | Small Intestine | Surface area of single villus = 0.96 $mm^2$<br>Mucosal surface area = 23 $cm^2/cm^2$ serosal surface area. |
| Mall (1887) | Dog | Fixed specimens | Small Intestine | Total mucosal surface area excluding the villi and crypts = 657 $cm^2$ |
| Krause (1879) | Man | Fixed specimens | Small Intestine | Surface area of single villus = 0.3-0.7$mm^2$<br>Mucosal area of whole small intestine = 1.6 sq. metres.<br>Ratio $\frac{\text{mucosal area}}{\text{serosal area}} = \frac{5}{1}$ |
| Reid, E.W. (1900) | Dog | Gelatine cast | Ileum and Colon | Mucosal surface area of the ileum = 6 sq. cm/unit serosal length.<br>Colon: mucosal surface area = 9$cm^2/cm^2$ serosal surface area. |
| Krogh (1922) (from Mall(1887) | Dog | Fixed specimens and calculations from Mall's Data | Not Specified | 7 $mm^2$ for each $mm^2$ serosal surface area. |
| Vimtrump (quoted by Krogh, 1922) | Rabbit | Fixed specimens | Duodenum | 17.6 $mm^2$ for each 1 $mm^2$ serosal surface |
| Vetzar (1936) | Pigeon | Direct measurements in the living. | Small Intestine | Mucosal surface area = 4.5 sq. metres. |
| Warren (1939) | Dog | Fixed Specimens | Small Intestine | Mucosal Surface = 1.6 sq. metres of which 7% was in the duodenum. |

## II METHODS.

It is very probable that the post-mortem length of the small intestine differs from the length during life (Espé and Cannon, 1932: 1940). Moreover, it is notorious that tissues fixed for histological work undergo distortion. Previous workers, too, seem to have made little attempt to ensure that the gut was fixed under standard conditions of distension. Warren (1939) was probably more aware of these various difficulties than his predecessors in this field. His method, therefore, has been used with several modifications in this present work.

The arrangement, shown in Fig. I, was employed. The animal, rat or cat, having been starved for the previous 24 hours, was anaesthetised with ether and the abdomen widely opened. A cannula, directed caudad, was tied into the proximal jejunum and an opening made in the ileum just craniad to the large intestine. The whole small intestine was then washed free of faecal matter by means of a stream of Ringer solution at 38°C. Thereafter, a second cannula, directed craniad, was tied into the intestine through the opening in the ileum. Midway between these two cannulae a piece of intestine was cut completely out and the remaining portions of the ileum and jejunum connected by tying in a T - piece of glass tubing. Attached to the upright limb of the T - piece was a length of rubber tubing. The length of this tubing determined the head of pressure to which all portions of the gut were uniformly exposed. The gut was then freed from the mesentery and all portions of the gut attached to the cannulae transferred to a large flat dish. Ringer solution was once more run through the gut entering

## Fig. 1.

Fig.1. shows diagrammatically the arrangement employed to fix the gut under uniform pressure so that measurement of surface area may be comparable.

by both cannulae and leaving by the vertical limb of the T-piece. Fixative in gradually increasing strength was then perfused through the gut and also poured over the serosal surface. The gut was thus rapidly fixed in a distended state under a known head of pressure, 7 cms. in the case of the rat and 15 cms. in the cat.

When fixation was complete straight portions of jejunum and ileum roughly 6 cms. in length were cut off. Each of these pieces was further divided into three smaller portions, a centre piece approximately 4 cms. long and two end pieces.

The large intestine was treated in a somewhat similar fashion, the only real difference being that the T-piece for regulating the head of pressure was unnecessary. Portions of intermediate colon were chosen for study.

In spite of all care and of rapid fixation, the epithelium of the small intestine was very apt to desquamate under the head of pressure. Several fixatives were tried with varying degrees of success. For the small intestine a solution of picric acid in dioxan (Carleton, 1938), was the most satisfactory; with the large intestine, Susa's fixative gave the best results.

After embedding in paraffin, transverse sections were cut from the end pieces and longitudinal sections were cut from the central portion at its greatest diameter (Fig.II). The sections were stained with Erlich's acid haematoxylin and Orange G. Typical sections from jejunum and ileum of the rat are shown in Figs. 3, 4, 5, and 6.

Fig. 2.

Fig. 2 shows diagrammatically how each portion of intestine was roughly divided into 3 segments after fixation in picro-dioxan. From the central portion longitudinal sections were cut, while from the other two portions, transverse sections were cut. In this way, data were obtained which allowed the mucosal surface area of the small intestine to be calculated.

To find the area of the mucous membrane the formula recommended by Warren (1939) was used.

$$\frac{EA}{SA} = \frac{MC}{SC} + \frac{ML}{SL} - 1$$

EA is the unknown, the estimated mucosal area.

SA is the serosal area.

MC is the mucosal circumference.

SC is the serosal circumference.

ML is the mucosal length.

SL is the serosal length.

To facilitate measurement, the sections were projected on to large sheets of white paper at known magnification varying between 70 and 90 diametres according to the size of the section. The mucosal and serosal outlines were drawn in pencil and then measured by a rotometer calibrated in centimetres. The values thus obtained, after due allowance for magnification, were then inserted in the formula and the unknown, EA, calculated. About 800 sections of gut in all were cut and stained.

## III. RESULTS.

### a) Histological Appearances.

In the same animal, the final microscopic preparations showed no significant difference between the diameter of the jejunum and of the ileum. In the rat, the average diameter was 0.5 cm., in the cat 1.1 cm. By simple inspection of Figs. 3, 4, 5, 6, it is obvious that in the rat the villi are more numerous in the jejunum than in the ileum. This is equally striking in the small intestine of the cat.

## Figs. 3 and 4.

Fig. 3 shows the appearance of a transverse section of a typical region of the jejunum of the rat. Since the villi of the rat are leaf-shaped, in transverse sections the villi are rather like mounds than long processes. The jejunum has obviously more villi than the ileum.

Fig. 4 shows the appearance of a transverse section of a typical region of the ileum of a rat.

## Figs. 5 and 6.

Fig. 5 shows the appearance of a longitudinal section of a typical region in the jejunum of the rat. Cut longitudinally, the leaf-shaped villi found in rat gut appear to be finger-like processes. Simple inspection shows that the villi in the jejunum are more numerous than in the ileum.

Fig. 6 shows the appearance of a typical region in the ileum of the rat.

In the rat the villi are leaf-shaped lying parallel to one another, the long axes of the villous leaves running at right angles to the length of the gut. In the cat the villi are long and finger-like.

b) The Area of the Mucous Membrane.

Measurements were carried out on eight rats and four cats. In Table II figures are given for the actual mucosal area per centimetre serosal length of the gut in the fixed state. Both in the rat and in the cat the mucosal area per unit length of gut is roughly 1½ times greater in the jejunum than in the ileum. This difference could obviously be due to greater gut diameter in jejunum than in ileum. Histologically, there was no evidence of this.

Between rat and cat there is a most striking difference in mucosal area per unit length of gut (Table II). Part of this difference is undoubtedly due to the greater diameter of the cat's intestine. This factor of gut diameter can be eliminated by finding the mucosal area per unit serosal area of the gut. This has been done in Table III by recording the ratio $\frac{\text{mucosal area}}{\text{serosal area}}$. This ratio indicates the ~~Serosal area~~ degree of villous development being $\frac{1}{1}$ in the absence of villi, as it is in the colon and increasing in magnitude with increasing villous development.

Table III shows clearly that the difference between jejunal and ileal mucosal area between rat and rat and cat and cat is almost wholly dependent on the number and possibly length of the villi. From Table III, it is also obvious that villous development is more marked in the cat than in the rat. As one would expect,

TABLE II.

Comparison between Mucosal Area in Jejunum and Ileum of the Rat and Cat.

Mucosal Area in Sq. Cm per 1 cm. Serosal Length.

| Animal. | Jejunum | Ileum. |
|---|---|---|
| Rat | | |
| 1 | 7.7 sq. cms. | 2.9 sq. cms. |
| 2 | 8.0 sq. cms. | 6.2 sq. cms. |
| 3 | 7.0 sq. cms. | 5.7 sq. cms. |
| 4 | 9.2 sq. cms. | 4.9 sq. cms. |
| 10 | 10.4 sq. cms. | 6.0 sq. cms. |
| Mean | 8.5 (± 1.90) | 5.1 (± 1.96) |
| Cat | | |
| 1 | 52.7 sq. cms. | 42.0 sq. cms. |
| 2 | 48.6 sq. cms. | 30.2 sq. cms. |
| 3 | 47.2 sq. cms. | 34.4 sq. cms. |
| Mean | 49.5 (± 1.65) sq. cms. | 35.5 (± 3.31) sq. cms. |

TABLE III.

| Animal | The Ratio $\frac{\text{Mucosal Area}}{\text{Serosal Area}}$ | | |
|---|---|---|---|
| | Jejunum | Ileum | Colon |
| Rat | $\frac{6}{1}$ | $\frac{4}{1}$ | $\frac{1}{1}$ |
| Cat | $\frac{15}{1}$ | $\frac{12}{1}$ | $\frac{1}{1}$ |

TABLE IV.

| | Mucosal Area in sq. cm. per 1 cm. | Serosal Length. |
|---|---|---|
| Animal | Ileum | Colon |
| Rat | 5.1 sq. cm. | 2.2 sq. cms. |
| Cat | 35.5 sq. cm. | 5.6 sq. cms. |

the intermediate colon has a much smaller mucosal area per unit length than the small intestine. This contrast is very striking in the cat (Table IV). Without villi the ratio $\frac{\text{mucosal area}}{\text{serosal area}}$ should be $\frac{1}{1}$ as was actually found in the colon.

(Table III)

The marked difference between mucosal area in jejunum and in ileum makes it extremely difficult to give a figure for the total absorbing surface of the small intestine even under standard conditions. Post-mortem, the average length of the small intestine of an adult rat is 100 cm. The length of the small intestine of an adult cat is not dissimilar.

Accepting these lengths, and assuming uniform decrease in mucosal area from jejunum to distal ileum, the figures for mucosal area per cm. length, Table II, indicate that the total mucosal area of the small intestine in the adult cat is in the neighbourhood of 4,300 sq. cm. The difference is most striking when it is remembered that the post-mortem length of the small intestine is little different from that of the adult rat.

## IV DISCUSSION

a) Absolute Values.

The absolute values in the present work are obviously valid only for gut subjected to the procedure here described. Nevertheless it is interesting that Warren (1939) found the mucosal area per centimetre serosal length to be 54 sq. cm in the jejunum of the dog and 38 sq. cm in the mid-ileum. Unfortunately, Warren's results were obtained from

experiments on one dog only. He admits, moreover, that the distal ileum was in spasm not overcome by the fixative and therefore the values which he obtained for this segment were not comparable with values obtained from segments in which distension was obtained with the fixative.

Acceptance of such data and indeed of all such figures available at the present time as a reliable basis for calculating the total mucosal surface of the intestine in vivo is greatly to be deprecated. Not only does histological treatment cause gross distortion but evidence is accumulating that in life the entire gut is much shorter than it is post-mortem. (Espé and Cannon, 1932;1940). However, Johnston (1913) showed that distension of the small intestine in guinea-pigs made the long finger - like villi become stumpy and much shorter. Thus it is just possible that the total mucosal area may be less affected by alteration in gut length and by distension than one would at first sight believe.

Estimation of the mucosal area of the small intestine in man is still further complicated by the existence of the valvulae conniventes. Some authorities believe that these mucosal folds greatly increase the mucosal area.

B. Relative Values.

Every care was taken to ensure that the whole small intestine in each animal reacted uniformly throughout its length to the manipulations of fixation and embedding. Distortions of the absolute values should then cancel out in making comparisons between different portions of the gut in the same animal and the comparisons should be valid for the living state.

It would appear that the mucosal surface of the jejunum per unit length in both rats and cats is roughly 1½ times as great as the mucosal surface of the ileum. This must be attributed in both animals to greater villous development in the jejunum since the diameter of the small intestine remained uniform throughout its length.

If we assume that the intestine of both rats and cats retained their relative dimensions throughout the processes of fixation and embedding then it is justifiable to compare the small intestine of the rat with that of the cat.

Villous development as a whole must be more marked in the small intestine of the cat than in the rat. The mucosal area per sq. cm. serosal area was roughly three times as great in the cat.

It has already been noted that the post-mortem lengths of the small intestines in both rats and cats were in the neighbourhood of 100 cm. Assuming a uniform decrease in mucosal area from cranial to caudal end of the small intestine it is possible to find the ratio,

$$\frac{\text{total mucosal area of the small intestine of the rat}}{\text{total mucosal area of the small intestine of the cat}}$$

from figures previously obtained. The average mucosal area per

centimetre length is 6.8 sq. cm. in the rat and 42.5 sq. cm. in the cat. This gives a ratio of $\frac{1}{6.3}$ implying that the mucosal area of the small intestine of the cat is more than 6 times the mucosal area of the small intestine in the rat.

The rats used in this work had an average weight of 295 gms., the cats an average body weight of 1,950 gms. The ratio, $\frac{\text{body weight of rats}}{\text{body weight of cats}}$ is therefore $\frac{1}{6.6}$

Without stressing unduly, in view of the assumptions involved, the close agreement between these two ratios, it is yet obvious that the mucosal surface of the small intestine has an intimate relationship to the body weight even when comparing two such different creatures as the rat and the cat. This relationship, if expressed in the form, $\frac{\text{mucosal surface of the small intestine}}{\text{body weight}}$ is a constant, immediately brings to mind a similar generalisation by Cori (1925). In his case, the result was perhaps not so unexpected since he worked only on rats of different weights. From the quantities of various sugars absorbed from the intestine he concluded that $\frac{\text{intestinal absorbing surface}}{\text{body weight}}$ is a constant body weight.

Thus, purely morphological observations in this thesis suggest that an important generalisation may be still further extended.

## V. SUMMARY.

A table summarising the previous data on the area of the intestinal mucous membrane in various animals is given at the beginning.

Rapid fixation and uniform distension of the gut in rats and in cats was ensured by running fixative through the lumen at a constant head of pressure. After embedding in paraffin, transverse and longitudinal sections of the gut were cut and stained. From measurements on these sections, magnified and projected, the area of the mucous membrane was calculated in jejunum, ileum and colon.

Both in the rat and cat the mucosal area per unit gut length is much greater in the jejunum than in the ileum. The ratio $\frac{\text{mucosal area}}{\text{serosal area}}$ is greater in the jejunum than in the ileum in both species. In the cat this ratio is nearly 3 times as great as the ratio in the rat indicating a greater development of villi in the cat.

In spite of marked difference in size and in habit, the total area of the mucous membrane in the entire small intestine bears a similar relation to body weight in the cat as in the rat.

## SECTION B.

Relative Rates of Absorption of Monosaccharides from Jejunum and Ileum of Rats under Urethane Anaesthesia.

I. Introduction.

II. Past Work.

III Present Work.

a) Method.

b) Results.

c) Discussion.

IV. Summary.

## I. INTRODUCTION

It was postulated by Verzâr (1936) that the difference in the rate of absorption of hexoses e.g. glucose, and pentoses, e.g. xylose, in the small intestine of the rat is due to a special activity of the intestinal mucosa, namely phosphorylation of the hexoses. He also stated that this special activity whereby glucose was preferentially absorbed, was particularly marked in cranial regions of the small intestine in comparison with caudal regions (Verzâr and Witz, 1937).

As shown in the previous section the mucosal area is less in the distal than in the cranial region of the small intestine. This factor will naturally not be without influence on absorption rates measured by the usual technique. Verzâr took no precautions to allow for this factor. The greater phosphorylation activity craniad in the gut may simply be an expression of greater mucosal absorbing surface.

However, should Vêrzar's postulated greater phosphorylating activity really exist in the cranial regions of the small gut, then the absorption rates of glucose and of galactose per unit area of mucosal surface should decrease from jejunum to ileum. On the other hand, xylose, said not to be phosphorylated in the process of absorption (Verzâr, 1936) should have the same rate of absorption per unit area mucosal surface both in jejunum and ileum since xylose is believed to be absorbed by simple diffusion. Any significant difference between the rates of absorption in jejunum and ileum

should, therefore, according to Verzar's hypothesis lie with glucose and galactose rather than with xylose.

## II. PAST WORK.

Höber (1898, 1899, 1903) found that in the intestinal loops of anaesthetised dogs some sugars were absorbed in proportion to their diffusion velocities i.e. monosaccharides more quickly than disaccharides. He did not take into account the probability that the slower rate of absorption of the disaccharides was due to the time taken by hydrolysis of these sugars into simpler monosaccharides before absorption could occur. He also discovered that glucose and galactose, in anaesthetised dogs, were absorbed at equally rapid rates from the small intestine. Hèdon (1900) tested the absorption rate of sugars from the small intestine of unanaesthetised rabbits. He used looks of gut separated from the small intestine leaving the mesenteric connections intact. Glucose was found to be absorbed more rapidly than galactose which in turn was more rapidly absorbed than arabinose. In 1902, Magano, comparing solutions of different concentrations in anaesthetised dogs, found that galactose was absorbed more rapidly than fructose. Fructose was found to be absorbed at a greater rate than xylose and arabinose.

Hewitt (1924) found in unanaesthetised rabbits and pithed cats, that glucose is more rapidly absorbed than galactose or fructose.

By feeding different sugar solutions to unanaesthetised rats by means of a stomach tube Cori (1925) established the relative rates of absorption in the intestine. Cori's work is so fundamental that the results of his experiments are given in detail.

Relative Rates of Absorption in the Unanaesthetised Rat (Cori)

| Sugar | Rate of Absorption |
|---|---|
| d - galactose | 110 |
| d - glucose | 100 |
| d - fructose | 43 |
| d - mannose | 19 |
| l - xylose | 15 |
| l - arabinose | 9 |

In addition, Cori made the generalisation that sugar absorption is, within wide limits, independent of the concentration of the solution introduced into the gut, in other words, sugar absorption is a function of the absorbing surface and largely independent of the solute concentration.

Cori's results were open to the objection that spasm of the pyloric sphincter might easily delay passage of the sugar solution from the stomach into the small intestine. Therefore, Verzár and his co-workers injected blood - isotonic solutions of the sugars directly into the intestinal loop thus overcoming this variable factor which may account for the slight discrepancies, between Cori's results and those given below.

Relative Absorption Rates in Anaesthetised Rats (Verzár)

| Sugar | Rate of Absorption |
|---|---|
| galactose | 115 |
| glucose | 100 |
| fructose | 44 |
| mannose | 33 |

Relative Absorption Rates in Anaesthetised Rats (Verzár) Contd.

| Sugar | Rate of Absorption |
|---|---|
| sorbose | 30 |
| xylose | 30 |
| arabinose | 29 |
| rhamnose | 29 |

These results were established by Wilbrandt and Laszt (1933) and later confirmed by Verzár (1935) and Laszt (1935).

McCance and Widdows (1930) found in man and rats (both unanaesthetised) that xylose was absorbed more rapidly than arabinose and rhamnose. Experiments by McLeod, Magee and Purves (1930) on cats and unanaesthetised rats with mixtures of sugars showed that, using the Cori technique, glucose was more quickly than any other sugar from mixtures of glucose and xylose or glucose and arabinose; and similarly galactose was more quickly absorbed than fructose from a mixture of these two sugars. They showed that the rates of absorption were in the same ratio when the sugars were mixed in solution as when they were separate. They did not, however, show whether the rate of absorption of each sugar was decreased by the presence of the other.

Auchinachie, McLeod and Magee (1930) suspended loops of small intestine in warm Mammalian Ringer in a Burn-Dale apparatus and allowed various sugars, placed in the lumen of the gut to diffuse through the mucosa, through the muscular coats and peritoneum to the Ringer bathing the outer coat of the gut. Since a known amount of sugar was placed in the lumen of the gut and the amount which had diffused out into the Ringer could be estimated, all the data were available for measuring

the rate of the diffusion. This method, however, did not measure rates of absorption since diffusion of sugars through the gut wall from the lumen to the exterior cannot by any stretch of imagination be said to be true absorption. Auchinachie, McLeod and Magee showed by this method that glucose diffuses out more rapidly through dead than living segments of excised loops of rabbits intestine. In surviving loops, glucose passes out more rapidly than xylose. On the other hand, if the loops are killed, xylose diffuses out at a slightly greater rate than glucose. If the temperature is reduced to 0°C the temperature at which metabolic activities are inhibited, glucose and xylose diffuse out at the same rate.

The first researches upon relative rates of absorption between upper and lower regions of the small intestine were conducted by Nagano (1902), Röhmann and Nagano (1903), Omi (1909), and Frey (1909) who showed that the absorption of glucose was greater in the duodenum and jejunum than in the ileum. King, Arnold and Church (1922) found that the absorption was greatest in the jejunum of dogs and rather less in the duodenum and ileum. London and Polowzowa (1906, 1908) stated that concentrated sugar solutions are diluted in the higher parts of the intestine and that absorption begins only at the ileal end. Auchinachie, McLeod and Magee (1930) found that diffusion of solutes out of surviving loops of rabbits intestine was more rapid towards the ileal end. Verzár and Wirz (1937) stated that the preferential absorption of glucose decreases as one progresses towards the caudal end of the small intestine. In 1940, Davidson

and Carry found that, within the caudal half of the small intestine of the cat there was no evidence for decreasing absorptive power for glucose in the more distal region. The table below, taken from Davidson and Carry (1940) and with some modifications, summarises the data on the relative rates of absorption from the small intestine.

TABLE V.

Relative Rates of absorption of monosaccharides from the small intestine

| Author | Animals | Relative Rates of Absorption |
|---|---|---|
| Höber (1899) | Dogs unaesthetised | Galactose = glucose |
| Hédon (1900) | Rabbits | Glucose > galactose > arabinose > raffinose |
| Nagano (1902) | Dogs unaesthetised | Galactose > glucose > fructose > mannose > xylose > arabinose |
| Hewitt (1924) | Rabbits unaesthetised | Glucose > fructose |
| | Cats pithed | Glucose > galactose > fructose |
| Cori (1925) | Rats unaesthetised | Galactose > glucose > fructose > mannose > xylose > arabinose. |
| McCance & Maddern (1930) | Man and rat unaesthetised | Xylose > arabinose > rhamnose |
| Auchinachie, Macleod & Magee (1930) | Rabbits isolated loops. | Glucose > xylose. |
| MacLeod, Magee and Purves (1930) | Rabbit and cat isolated loops. | Glucose > xylose |
| | Rats unaesthetised | |
| Smalie & Hendry (1932) | Chickens | Glucose > galactose |
| Burget, Moore Lloyds, (1932) | Dogs, rabbits, rats | Glucose > fructose (difference small) |

TABLE V. (Contd.)

Relative Rates of absorption of monosaccharides from the small intestine.

| Author | Animals | Relative Rates of Absorption |
|---|---|---|
| ~~Lloyd (1932)~~ | ~~Rats~~ | |
| Miller & Lewis (1932) | Rats unanaesthetised. | Glucose > xylose |
| Wertheimer (1932) | Rats | Galactose > glucose > fructose |
| Wilbrandt & Laszt (1933) | Rats "Numal" and urethane | Galactose > glucose > pentoses |
| Cajori & Karr (1935) | Dogs | Galactose = glucose. |
| Westenbrink (1936b) | Rats unanaesthetised. | Galactose > glucose > fructose > mannose > xylose > arabinose. |
| | Pigeon urethane | Galactose > glucose > fructose > mannose > xylose > arabinose |
| Roberts, A. (1936) | Dogs unanaesthestised. | glucose > sucrose. |
| Westenbrink & Gratama (1937) | Frogs anaesthetised | Galactose > glucose > mannose > fructose > xylose > arabinose |
| Groen (1937) | Man unanaesthetised | Galactose > glucose > fructose |
| Klinghoffer (1938) | Rats unanaesthetised | Glucose > xylose |
| Davidson & Carry (1939) | Rats urethane | Galactose > glucose > fructose > xylose. |

## III. PRESENT WORK.

a) Method.

The method was basically that of Cori (1925) in that a known quantity of a sugar solution of definite strength was placed in the gut and the quantity remaining determined after a time interval. The rats were all kept on a complete stock diet of rat cubes (Thomson, 1936), milk and greens since there is little doubt that the previous dietetic history may affect the absorbing power of the gut (Westenbrink, 1934). For the 24 hours previous to the experiment, the rats received only water.

They were then given a subcutaneous injection of 25% urethane (ethyl carbamate) solution which had been freshly made. The dosage was 1.6 mgms urethane per gramme rat. In some cases it was necessary to increase the dose slightly to obtain the required depth of anaesthesia. Usually, sufficient depth of anaesthesia was reached an hour after the injection of urethane. During this period the animal was kept at a moderately warm temperature, e.g., in an animal cage over a warm radiator. Rats, under urethane anaesthesia, seem to be very sensitive to environmental temperature, rather unexpectedly to high temperatures. It was found that, if at this stage the animal was placed on a previously warmed operating board for the time required to induce anaesthesia, the animal suffered from hyperthermia, showed signs of cyanosis and respiratory distress. Death very often ensued. Urethane, in smaller dose, was the anaesthetic used by Verzár and McDougall (1936) whose absorption values from the small intestine in normal rats agree

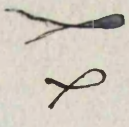
well with those of Cori (1925) on unanaesthetised rats. The dose of urethane employed by Verzár was 1.2 mgms urethane 100 gms body weight rat was found to be insufficient to induce full anaesthesia. It has been shown that urethane has a comparatively slight inhibiting influence on the absorption of water from the gut (Heller and Smirk, 1932).

The abdomen was opened one hour after the injection of urethane and the small intestine from the lower ileum to the upper jejunum exposed.

A ligature A (see Figure 7) was tied round the extreme upper end of the jejunum. A second ligature B was placed at a point about 30 centimetres caudal to A. The ends of this ligature were not tied but left loose. A snip, sufficient to accommodate a cannula, was made between A and B. To ensure approximately equal weights and lengths of the upper and lower loops of the small gut, a piece of string 20 centimetres long and of medium diameter was used to measure off a length of gut from B. A ligature C, similar to B, was placed round this point which was approximately 20 centimetres caudal from B. A ligature D, corresponding to A, was tied round the jejunum at a point approximately 3 centimetres caudal to C. A snip through the gut was made between C and D. Similarly beginning at the caecum, ligatures G and H were placed round the ileum 3 centimetres apart H being tied but G left loose. Twenty centimetres of gut from G to F were measured with the string E and F represent the other two necessary ligatures - E being tied and F left loose. A snip big enough to admit a

Fig. 7.

Fig. 7 shows the position of the ligatures on the gut of an anaesthetised rat during a routine absorption experiment to determine the relative rates of absorption of monosaccharides from jejunum and ileum respectively.



Ligature tied at once

Ligature left loose initially.

cannula was made through the gut wall between E and F another snip cut between G and H. A cannula was then placed into the opening between C and D, and E and F. All faecal matter was washed out with warm mammalian Ringer at 37°C.

Excess Ringer was removed by introducing another cannula into each loop and puffing air very gently through the lumen of the gut.

The loose ligatures G and B were then tied off. 1.5 mls of a blood isotonic sugar solution at 37°C were run into each loop by means of a cannula from a microburette. The segments were tied off, the abdomen sewed up and the animal left in warm surroundings for 40 minutes in the case of glucose and galactose and 60 minutes in the case of fructose and xylose. The animal was then opened up again and killed by making a snip in the heart and bleeding the animal to death Each loop was carefully removed from the abdomen when removing the mesentery care was taken to avoid stretching the loops. A small opening was made in each loop just below the upper ligature and another just above the lower. A cannula was introduced into the upper opening and the contents of the gut washed into a medium - sized funnel drainage into a 25 c.c. graduated flask. The contents of the flask were made up to the mark and the sugars estimated in duplicate using the method of Hagedonn and Jensen. A control, in which 1c.c. of blood isotonic sugar solution was diluted in 25 ccs, was estimated simultaneously with the duplicate estimations.

Immediately, the loops had been washed out, they were measured by placing them vertically against a fixed scale and the length determined in centimetres. Excess moisture was removed from them

by filter paper and the loops weighed.

The sugars used were d-glucose, d-galactose, d-fructose and d-xylose ( $[\alpha]_D$ + 19.5°) supplied by British Drug Houses. Solutions were made up 24 hours before use. In the case of hexoses, the concentration of sugar solutions was 5.4%; in the case of pentoses the concentration of sugar solution used was 4.5%. These are the usually accepted values for blood isotonicity.

b). Results.

The results of each experiment were recorded in a protocol as in Table VI.

In Tables VII, VIII, IX, XI and XI all the data necessary for the determination of the relative absorption values in jejunum and ileum of the rat are recorded. Table VII records the results obtained with glucose in 9 rats. In six of these the time of absorption was 40 minutes and in five the data necessary to estimate the mucosal area of jejunal andileal loops were obtained. Table VIII shows the results obtained with galactose in 11 rats. In four of these the time of absorption was 40 minutes and in four the data for estimating the jejunal mucosal area and the ileal mucosal area were determined.

Table IX gives the results for the absorption of fructose in 8 rats. The time of absorption was 60 minutes in all cases but the results were corrected for an absorption time of 40 minutes. Similarly, Table X shows the absorption values for xylose in 9 rats and in five of these the mucosal area of the loops has been calculated. The time of absorption was 60 minutes but the results were corrected for an

TABLE VI

Typical protocol used in recording results.

Rat serial number 33 (white) Date 10/1/41.

Sex Male Weight 190 gms.

Pre-experimental treatment Water only for 24 hours

Anaesthetic Urethane Dose 1.6 mgms. per. gm. rat

Substance tested Glucose

Concentration 5.4%

Volume 1.5 mls. per loop

Time of absorption 40 mins.

Region of gut Cranial weight 1.37 length 18.1 cms.

Caudal weight 1.25 length 14 cms.

| Region | Initial | Final | Difference | mgms. absorbed p. gut |
|---|---|---|---|---|
| Cranial | 81 | 27.35 | 53.65 | 39.1 |
| Caudal | 81 | 38.25 | 43.75 | 35.0 |

Comments:- .2 ccs. additional urethane given.

mgms. absorbed / cm. gut

2.96

3.13

Condition of animal:- good.

absorption time of 40 minutes.

It will be noticed in the column recording the volume of galactose solution placed in each loop that in early experiments 2 c.cs of sugar solution per loop were used whereas in later experiments the amount of solution placed in each loop is reduced to 1.5 mls. In the initial experiments the whole small intestine from the beginning of the jejunum to the caecum was roughly divided into two portions and 2 mls of sugar solution placed in each loop. It was then decided to use a typical piece of jejunum by taking a smaller loop of gut than that previously used but still adjacent to the duodenum. Similarly a small piece of ileum adjacent to the caecum was used in order to obtain a typical portion of ileum. A fairly long piece of gut between the jejunal and ileal loops was therefore left untouched. This is represented in Figure 7 by DE. To avoid undue distension in these smaller loops 1.5 mls of sugar solution was introduced into each loop instead of 2 mls.

The time allowed for absorption is obviously a factor of no little moment. Ideally, the duration should be such that the rat has full opportunity to recover from the disturbances due to laparotomy and insertion of the sugar solution. In addition the time should be of such length that absorption is fully under way but should not be so long that practically all the sugar is absorbed. For example, choice of too long an absorption period may give the impression that two sugars have equal absorption rates if the time is sufficient to allow practically 100% of the more slowly absorbed sugar to disappear from the gut lumen. From the technical point of view, moreover, estimation of minute

quantities of monosaccharides is undesirable.

For no very obvious reason Cori and Verzár and his co-workers chose an absorption period of 1 hour for all sugars. Preliminary experiments convinced me that not far short of 100% of both glucose and galactose could be absorbed in some shorter period, say 55 minutes. For this reason, an absorption period of 40 minutes was chosen for both glucose and galactose. Such an interval allows fully half the sugar to disappear from the gut lumen. With such slowly absorbed sugars such as fructose and xylose on the other hand, 40 minutes was too short a period. 60 minutes was retained as absorption period with these two sugars.

The results, however, in the case of xylose and fructose were reduced to the common time of 40 minutes, the time which was found to be most suitable in the case of galactose and glucose since it is necessary to have such a common time before the relative rates of absorption of glucose, galactose, fructose and xylose can be compared. Taking the absorption rate values of xylose and fructose for 60 minutes, these results were multiplied by 2/3 thus giving the absorption values for an absorption time of 40 minutes on the assumption that absorption proceeds uniformly throughout the period.

The column under the heading "actual absorption" represents the amount of sugar which had disappeared from the gut lumen. To obtain the absorption rate per unit weight gut the actual absorption in mgms. from each loop was divided by the weight of the loop in gms. Similarly the absorption rate per unit length was obtained by dividing the actual absorption in mgms from each loop by the length of the loop in cms.

In the previous section, the mucosal area per unit serosal length in jejunum and in ileum was calculated. Knowing the length of each jejunal loop used, the mucosal area in sq. cms of these loops was calculated by multiplying the length by 8.5, the mucosal area per serosal unit length in the jejunum. Similarly, the mucosal area in sq. cms of the ileal loops was calculated by multiplying the length of the loop by the factor 5.1, the mucosal area per unit serosal length in the ileum. The absorption rate per sq. cm. jejunum was obtained by dividing the actual absorption by the mucosal area thus calculated. The absorption rate per sq. cm. ileum was found in the same way. These three methods of expressing the rate of absorption are tabulated in the three columns following the column recording the actual absorption. In the case of xylose and fructose, these results, corrected for a period of absorption of 40 minutes are given in Tables IX and X.

In Table XI, the average absorption values, the actual absorption the rate of absorption per unit weight the rate of absorption per unit length, the rate of absorption per unit surface area, over a period of 40 minutes in the case of glucose and galactose are recorded for jejunum and ileum in the rat. The average absorption values for fructose and xylose when the time of absorption is 60 minutes are given and also the average absorption values corrected to an absorption period of 40 minutes are shown.

TABLE VII

| Date | Animal | Initial vol. per loop. ml. | Time of absorption min. | JEJUNUM Weight gut g. | JEJUNUM Length gut cm. | JEJUNUM Absorption Actual mgm. | JEJUNUM Absorption per g. gut mgm. | JEJUNUM Absorption per cm. gut mgm. | JEJUNUM Absorption per sq. cm. gut mgm. | ILEUM Weight gut g. | ILEUM Length gut cm. | ILEUM Absorption. Acutal mgm. | ILEUM Absorption. per g. gut mgm. | ILEUM Absorption. per cm. gut mgm. | ILEUM Absorption. per sq. cm. gut mgm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 9/9/41 | Rat,M. | 1.5 | 90 | 2.0 | ---- | 80.1 | 39.3 | ---- | ---- | 2.6 | ---- | 77.7 | 35.9 | ---- | ---- |
| 13/11/41 | Rat,F. | 2.0 | 60 | 2.8 | ---- | 94.3 | 33.1 | ---- | ---- | 2.2 | ---- | 74.3 | 34.2 | ---- | ---- |
| 15/11/41 | Rat,F. | 1.5 | 60 | 1.7 | ---- | 74.0 | 41.4 | ---- | ---- | 1.4 | ---- | 45.8 | 32.5 | ---- | ---- |
| 18/11/41 | Rat,F. | 1.5 | 40 | 2.1 | ---- | 46.0 | 21.5 | ---- | ---- | 1.3 | ---- | 36.1 | 26.2 | ---- | ---- |
| 20/11/41 | Rat,F. | 1.5 | 40 | 1.7 | 19.0 | 76.3 | 44.6 | 4.0 | 0.5 | 1.2 | 12.0 | 56.8 | 47.0 | 4.7 | 0.9 |
| 6/ 1/42 | Rat,F. | 1.5 | 40 | 1.6 | 21.2 | 56.0 | 35.8 | 2.6 | 0.3 | 1.8 | 21.3 | 28.0 | 15.4 | 1.3 | 0.2 |
| 8//1/42 | Rat,M. | 1.5 | 40 | 1.5 | 16.0 | 57.8 | 38.7 | 3.6 | 0.11 | 1.6 | 18.3 | 27.8 | 17.6 | 1.5 | 0.3 |
| 10/ 1/42 | Rat,M. | 1.5 | 40 | 1.4 | 12.1 | 53.7 | 39.1 | 3.0 | 0.3 | 1.3 | 14.0 | 43.8 | 35.0 | 3.1 | 0.6 |
| 13/ 1/42 | Rat,F. | 1.5 | 40 | 1.3 | 18.4 | 39.5 | 29.6 | 2.2 | 0.3 | 1.3 | 15.3 | 38.7 | 30.4 | 2.5 | 0.5 |

GLUCOSE

TABLE VIII

| Date | Animal | Initial vol. per loop ml. | Time of absorption. min. | JEJUNUM Weight gut g. | JEJUNUM Length gut cm. | JEJUNUM Absorption Actual mgm. | JEJUNUM Absorption per g. gut mgm. | JEJUNUM Absorption per cm. gut. mgm. | JEJUNUM Absorption per sq. cm. gut mgm. | ILEUM Weight gut g. | ILEUM Length gut cm. | ILEUM Absorption Actual mgm. | ILEUM Absorption per g. gut mgm. | ILEUM Absorption per cm. gut. mgm. | ILEUM Absorption per sq. cm. gut. mgm. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 23/9/41 | Rat, M. | 2.0 | 60 | 3.4 | ----- | 95.1 | 28.3 | --- | --- | 2.1 | ----- | 84.9 | 40.8 | --- | --- |
| 28/9/41 | Rat, M. | 2.0 | 60 | 1.9 | ----- | 92.2 | 48.0 | --- | --- | 2.8 | ----- | 81.4 | 29.2 | --- | --- |
| 4/10/41 | Rat, M. | 2.0 | 60 | 3.0 | ----- | 105.8 | 35.4 | --- | --- | 2.0 | ----- | 94.3 | 48.3 | --- | --- |
| 9/10/41 | Rat, M. | 2.0 | 60 | 2.6 | ----- | 92.3 | 35.0 | --- | --- | 1.6 | ----- | 49.5 | 31.6 | --- | --- |
| 11/10/41 | Rat, M. | 2.0 | 60 | 2.7 | ----- | 100.1 | 38.1 | --- | --- | 1.3 | ----- | 74.6 | 58.3 | --- | --- |
| 19/10/41 | Rat, F. | 1.5 | 60 | 1.6 | ----- | 73.5 | 44.6 | --- | --- | 1.8 | ----- | 55.1 | 30.0 | --- | --- |
| 21/10/41 | Rat, F. | 1.5 | 60 | 1.9 | ----- | 59.5 | 32.0 | --- | --- | 1.3 | ----- | 36.0 | 28.0 | --- | --- |
| 24/11/41 | Rat, F. | 1.5 | 40 | 1.2 | 15.0 | 58.0 | 49.3 | 3.9 | 0.4 | 1.4 | 12.4 | 42.2 | 30.3 | 3.4 | 0.6 |
| 27/11/41 | Rat, F. | 1.5 | 40 | 2.1 | 16.8 | 76.6 | 37.0 | 4.5 | 0.5 | 1.6 | 12.2 | 43.7 | 28.1 | 3.5 | 0.7 |
| 1/12/41 | Rat, F. | 1.5 | 40 | 2.5 | 30.5 | 68.9 | 27.6 | 2.3 | 0.3 | 1.5 | 16.5 | 55.7 | 38.1 | 3.4 | 0.7 |
| 4/12/41 | Rat, F. | 1.5 | 40 | 1.2 | 16.5 | 39.9 | 32.5 | 2.4 | 0.3 | 1.0 | 11.4 | 55.7 | 54.6 | 4.9 | 0.8 |

GALACTOSE

TABLE IX.

| | | | | JEJUNUM | | | | | | ILEUM | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Absorption | | | | | | Absorption | | | |
| Date | Animal | Initial vol. per loop | Time of Absorption | Weight gut | Length gut | Actual | per g. gut | per cm gut | per sq cm gut | Weight gut | Length gut | Actual | per g. gut | per cm gut | per sq cm gut |
| | | ml. | min. | g. | cm. | mgm. | mgm. | mgm. | mgm. | g. | cm. | mgm. | mgm. | mgm. | mgm. |
| 23/10/41 | Rat, F. | 1.5 | 40 | 1.1 | ---- | 12.8 | 11.6 | --- | --- | 1.0 | ---- | 10.6 | 10.4 | --- | --- |
| 26/10/41 | Rat, F. | 1.5 | 40 | 1.4 | ---- | 8.5 | 6.2 | --- | --- | 0.9 | ---- | 10.5 | 11.7 | --- | --- |
| 29/10/41 | Rat, F. | 1.5 | 40 | 0.9 | ---- | 18.5 | 13.1 | --- | --- | 0.9 | ---- | 11.2 | 11.9 | --- | --- |
| 15/1/42 | Rat, M. | 1.5 | 40 | 0.9 | 13.7 | 17.1 | 18.3 | 1.3 | 0.1 | 1.4 | 13.9 | 19.7 | 14.1 | 1.4 | 0.3 |
| 17/1/42 | Rat, M. | 1.5 | 40 | 1.1 | 14.2 | 18.7 | 17.8 | 0.9 | 0.1 | 0.7 | 10.0 | 17.5 | 24.3 | 1.8 | 0.3 |
| 20/1/42 | Rat, M. | 1.5 | 40 | 1.7 | 19.1 | 35.0 | 20.5 | 1.8 | 0.1 | 1.3 | 16.2 | 19.0 | 14.4 | 1.1 | 0.2 |
| 24/1/42 | Rat, M. | 1.5 | 40 | 1.0 | 18.0 | 24.2 | 15.5 | 1.3 | 0.2 | 1.6 | 17.2 | 14.7 | 9.2 | 0.8 | 0.2 |
| 27/1/42 | Rat, M. | 1.5 | 40 | 1.0 | 12.0 | 10.3 | 9.4 | 0.9 | 0.1 | 1.5 | 16.3 | 13.6 | 8.9 | 0.8 | 0.2 |

FRUCTOSE

(Actual time of absorption - 60 minutes; results "corrected" to 40 minutes)

**TABLE X.**

| Date | Animal | Initial vol. per loop | Time of absorption | JEJUNUM | | | | | | ILEUM | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Absorption | | | | | | Absorption | | | |
| | | | | Weight gut | Length gut | Actual | per g. gut | per cm gut | per sq cm gut | Weight gut | Length gut | Actual | per g. gut | per cm gut | per sq cm gut |
| | | ml. | min. | g. | cm. | mgm | mgm | mgm | mgm | g. | cm. | mgm | mgm | mgm | mgm |
| 1/11/41 | Rat,F. | 1.5 | 40 | 1.0 | ---- | 18.7 | 18.7 | --- | --- | 0.8 | ---- | 13.3 | 16.7 | --- | --- |
| 4/11/41 | Rat,F. | 1.5 | 40 | 1.6 | ---- | 10.2 | 6.6 | --- | --- | 1.6 | ---- | 15.2 | 9.2 | --- | --- |
| 6/11/41 | Rat,F. | 1.5 | 40 | 1.1 | ---- | 12.5 | 11.4 | --- | --- | 2.1 | ---- | 18.2 | 8.5 | --- | --- |
| 8/11/41 | Rat,F. | 1.5 | 40 | 1.1 | ---- | 19.7 | 17.1 | --- | --- | 1.1 | ---- | 17.0 | 15.9 | --- | --- |
| 11/11/41 | Rat,F. | 1.5 | 40 | 1.5 | ---- | 16.2 | 10.5 | --- | --- | 1.0 | ---- | 10.0 | 10.3 | --- | --- |
| 31/1/42 | Rat,F. | 1.5 | 40 | 1.4 | 15.6 | 21.2 | 14.2 | 1.4 | 0.2 | 1.5 | 17.4 | 12.5 | 6.1 | 0.7 | 0.1 |
| 3/2/42 | Rat,M. | 1.5 | 40 | 1.6 | 15.1 | 25.3 | 16.1 | 0.7 | 0.2 | 1.6 | 18.0 | 11.0 | 6.9 | 0.5 | 0.1 |
| 7/2/42 | Rat,M. | 1.5 | 40 | 1.9 | 15.9 | 26.5 | 13.7 | 1.7 | 0.2 | 1.9 | 14.0 | 22.5 | 11.8 | 1.6 | 0.1 |
| 10/2/42 | Rat,M. | 1.5 | 40 | 1.5 | 19.8 | 25.7 | 16.7 | 1.3 | 0.2 | 1.6 | 19.3 | 8.8 | 5.5 | 0.5 | 0.1 |

**XYLOSE**

(Actual time of absorption = 60 minutes, results "corrected" to 40 minutes)

TABLE XI

## ABSORPTION

| Sugar used | Initial vol. per loop. ml. | Time of absorption min. | JEJUNUM Actual mgm | JEJUNUM per g. gut. mgm | JEJUNUM per cm. gut. mgm | JEJUNUM per sq. cm. gut mgm | ILEUM Actual mgm | ILEUM per g. gut. mgm | ILEUM per cm. gut mgm | ILEUM per sq. cm. gut. mgm | Comments |
|---|---|---|---|---|---|---|---|---|---|---|---|
| GLUCOSE | 1.5 | 40 | 54.9 | 34.9 | 3.03 | 0.36 | 38.5 | 28.6 | 2.64 | 0.50 | 6 results averaged |
| GALACTOSE | 1.5 | 40 | 60.9 | 36.6 | 3.26 | 0.37 | 49.3 | 37.8 | 8.79 | 0.70 | 4 results averaged |
| FRUCTOSE | 1.5 | 40 | 21.1 | 16.3 | 1.24 | 0.12 | 16.9 | 14.2 | 1.19 | 0.24 | 5 results averaged |
| XYLOSE | 1.5 | 40 | 24.7 | 15.2 | 1.27 | 0.20 | 13.7 | 7.6 | 0.83 | 0.10 | 4 results averaged |
| FRUCTOSE | 1.5 | 60 | 31.6 | 24.4 | 1.86 | 0.20 | 25.5 | 21.2 | 1.79 | 0.30 | 5 results averaged |
| XYLOSE | 1.5 | 60 | 37.0 | 22.8 | 1.91 | 0.25 | 20.6 | 11.4 | 1.25 | 0.75 | 4 results averaged. |

AVERAGE RESULTS.

(Results from the earlier experiments, and from all those not wholly free from technical fault, are omitted.)

c) Discussion

Once the relative mucosal area in jejunum and ileum had been obtained, the results were used to find the rates of absorption of glucose, galactose, fructose and xylose per sq. cm. mucosa in jejunum and ileum. These results are seen in Tables VII, VIII, IX and X, and brought together in Table XI. This is the ideal method of expressing rates of absorption since the mucosal area across which the diffusible substances pass is of fundamental importance. In the past, workers in this field attempted to standardise and compare their results by expressing the absorption rate of substances from the gut lumen in terms of quantity absorbed per unit gut length or gut weight or body weight or body surface area in unit time. These measurements were used on the assumption that gut length, gut weight, body weight and body surface area were functions of mucosal surface area of the gut. As far as I know no one has expressed the rates of absorption in terms of quantity absorbed per unit mucosal surface area. All methods of expressing rates of absorption without reference to the mucosal area suffer from one or more defects.

The measurement of the gut length involves obvious inaccuracies which cannot be avoided in view of the tortuous course followed by the gut and the attachment of the gut to the mesentery. Another method is to kill the animal, free the gut from the mesentery and suspend it against a vertical measure. Such a procedure, however, gives no true indication of the length of the gut in life. Reference has already been made to the work of Espé and Cannon (1932; 1940) on this

subject.

Presumably the weight of the gut will be directly proportional to the number of villi and therefore to the mucosal area of the gut if the other layers of the gut wall are also directly proportional to the weight of the gut. Theoretically, gut weight ought to be a more reliable measurement than gut length. Unfortunately, it is impossible to dry equally each piece of gut with filter paper and consequently the moisture content will vary and the gut weights will be inaccurate.

The reliability of body weight and body surface area as methods of expressing rates of absorption depends on the accuracy of the relationship between these measurements and the mucosal surface area of the gut. It is not convenient to use body weight and body surface area if loops of gut, and not the whole gut, are employed to determine the rate of absorption of a substance.

Before it is possible to compare the relative rates of absorption in the jejunum and ileum it is necessary to express these rates in terms of either unit gut length, unit gut weight or unit mucosal surface area. The former two were abandoned in favour of expressing the rates of absorption in terms of unit surface area. The only accurate means of expressing rates of absorption is in mgms. material absorbed per sq.cm. pre-mortem mucosal surface area. Since, however, the calculations of mucosal area per unit serosal length were made from sections of a post-mortem length of gut and applied to post-mortem length of gut and applied to post-mortem lengths of intestinal loops it may be assumed that any difference in the post-mortem length and

pre-mortem length will be the same in all cases. That is to say, if we have a pre-mortem length of gut of 5 cms. which becomes a post-mortem length of 10 cms. and another loop of pre-mortem length of 5 cms, post-mortem length 10 cms, which is fixed the relative mucosal area for the two pieces will be the pre-mortem and post-mortem. But if the post-mortem mucosal area per serosal length is determined in the fixed loop and applied to any other post-mortem loop of gut we are really finding the relative pre-mortem area of that loop. The gut may have changed its length after death but assuming that change of length is constant such alteration will not affect the relative mucosal areas of the loops. Should any unnatural stretching occur the mucosal area calculated for a loop of gut will be in excess of the pre-mortem mucosal area calculated for a loop of gut will be in excess of the pre-mortem mucosal area and an artificially low rate of absorption per unit surface area recorded. Such stretching, however, takes place in both jejunum and ileum presumably, though the relatively greater absorption in the ileum per unit surface area may be due to an artificially low rate in the jejunum caused by greater stretching of the jejunum. Such stretching however, could hardly affect the fact that the villi are certainly more numerous in the jejunum that in the ileum. When the upper and lower segments of the small intestine are compared therefore, it seems that the envons cancell out.

Considering the results in Tables VII to XI it is evident that galactose has the greatest rate of absorption in the small intestine no matter whether the rate of absorption is expressed in mgms galactose

absorbed per gm. gut, mgms galactose absorbed per cm. gut or mgms galactose absorbed per sq. cm. mucosal surface area. Glucose has a slightly lower rate of absorption while fructose and xylose are absorbed still more slowly - xylose being the slowest of all four sugars.

The relative rates of absorption of glucose in jejunum and ileum appear to be approximately equal - the average absorption rate of glucose is 34.9 mgms per gm. gut in the jejunum and 28.6 mgms per gm. gut in the ileum during an absorption period of 40 minutes. If however, the absorption rates are expressed in mgms glucose absorbed per unit surface area there is a definite difference in the rates of absorption in jejunum and ileum. The ileum absorbs glucose at a greater rate than the jejunum, the ileum absorbing 0.50 gms glucose per sq. cm mucosal surface area while the jejunum absorbed 0.36 mgms per sq. cm. mucosal surface area in 40 minutes.

In the case of galactose, the rate of absorption in the jejunum is slightly less than that in the ileum if the rates of absorption are expressed in mgms. per gm. gut. The rate of absorption in the jejunum is 36.6 mgms galactose absorbed per gm. gut. The time of absorption in both cases is 40 minutes. If the absorption rate is expressed in mgms galactose absorbed per unit surface area the greater rate of absorption in the ileum becoming more marked. The jejunum absorbed 0.37 mgms galactose per sq. cm mucosal surface area in 40 minutes while the ileum absorbed 0.70 mgms galactose per sq. cm. mucosal surface area in the same time.

Similar variations in the relative absorption rates of fructose in

jejunum and ileum occur. The absorption rate of fructose in the jejunum is 16.3 mgms per gm. gut and in the ileum it is 14.2 mgms per gm. gut. In the ileum there is a greater rate of absorption than in the jejunum if the rates of absorption of fructose are expressed per unit surface area. The jejunum absorbs fructose at the rate of 0.12 mgms per sq. cm. mucosal surface area gut in 40 minutes. The ileum absorbs fructose at the rate of 0.24 mgms per sq. cm mucosal surface area in 40 minutes.

On the other hand, xylose, which, according to Verzár's hypothesis, ought to be absorbed equally in the jejunum and ileum is absorbed more rapidly in the jejunum. The absorption rate of xylose in the jejunum is 15.2 mgms per gm. gut in 40 minutes; in the ileum the absorption rate is 7.6 mgms per gm. gut in 40 minutes. Even when the absorption rates are expressed in terms of mgms xylose absorbed per sq. cm mucosal surface area, the jejunum absorbs this pentose more rapidly than the ileum. The jejunum absorbs 0.20 mgms xylose per sq. cm. mucosal area while the ileum absorbs only 0.10 mgms xylose per sq. cm mucosal area in 40 minutes.

The present results, unfortunately, do little to explain the preferential absorption of monosaccharides. However, one fact is clear, the power to absorb monosaccharides per sq. cm mucosal area is not greater in the jejunum than in the ileum. This is rather surprising in view of the usual belief that the blood supply to the cranial portion of the small intestine is greater than that to the caudal portion. There is, at any rate, no justification for

postulating special elaboration of any "mechanism" phosphorylating or otherwise, for absorption of galactose and glucose in the cranial region of the small intestine.

Do the jejunum and ileum normally absorb carbohydrates? Abbot, Karr and Miller (1937) by a series of intubation studies on human subjects found that if a hypertonic solution of glucose was introduced into the stomach the duodenum was responsible not only for diluting the glucose until it was isotonic with the blood but for absorbing the greater part of the glucose so diluted and the jejunum and ileum were not called upon to absorb large quantities of carbohydrate.

They found that, when glucose is ingested even in large amounts, only a few grammes ever reach the jejunum. Shay, Gerahon, Cohen, Polo and Munro (1938) also state that the duodenum possesses a remarkable versatility in shifting from an absorptive organ for glucose in low concentrations coming from the stomach to a dilution organ for solutions of high concentration reaching it. They showed that, when hypertonic glucose meals are of concentrations not injurious to the duodenum the dilution mechanism of the duodenum assures a stream of glucose to the upper jejunum that is at or below isotonicity. Under such conditions the small intestine beyond the duodenum always acts in an absorptive capacity only. The distal portions of the small intestine, however, would be able to dilute hypertonic solutions should they reach the ileum. These workers also state that although the human stomach adds diluting fluids to ingested meals that are hypertonic it is not the important diluting organ. Jejunum and ileum can

therefore absorb carbohydrate but unless under special circumstances they are rarely called upon to do so. It does not necessarily follow that this impairs the ability of the ileum and jejunum to absorb carbohydrate under conditions allowing the absorption of carbohydrate in this regions.

The experiments of the workers quoted immediately above, however, are essentially abnormal and the implication that the jejunum and ileum are rarely called upon to absorb glucose by no means certain. Under normal conditions the bulk of carbohydrate food is not usually ingested as a solution of glucose. We have really little information about the rate of digestion of polysaccharides in food. It is quite conceivable that carbohydrate foods are usually well into the jejunum and even into the ileum before they are largely hydrolysed to absorbable monosaccharides. Thus the results I have obtained showing a relatively greater absorbing power in the ileum may be nearer the truth.

## IV Summary.

The relative rates of absorption of glucose, galactose, fructose and xylose in the jejunum and ileum of the rat were determined. These absorption rates were expressed in terms of mgms. sugar absorbed per gm. gut, mgms. sugar absorbed per cm. gut and mgms. sugar absorbed per sq. cm mucous membrane.

When the absorption rates were expressed in terms of unit gut weight and unit gut length glucose, galactose, fructose and xylose were all absorbed more rapidly in the jejunum than in the ileum. However, when the rates of absorption were expressed in terms of unit mucosal

surface area, glucose, galactose and fructose appeared to be more rapidly absorbed in the ileum than in the jejunum. On the other hand, xylose appeared to be more rapidly absorbed in the jejunum than the ileum.

There was little evidence, therefore, that the phosphorylating mechanism was more active in the jejunum than the ileum.

# SECTION C.

Rate of "Absorption" of Glucose and Xylose from the Intestine of Dead Rats.

Rate of "Absorption" of Glucose and Xylose from the Intestine of Dead Rats.

## I. Introduction.

From time to time, work has been carried out to measure the rate of absorption of substances from the intestine of dead animals. Under such conditions, a surprisingly large amount of the solute in the gut lumen will disappear presumably into the substance of the gut. Many of the earlier investigators carried out such absorption experiments upon dead cats and dogs. Waymouth Reid (1892-1902) has some extremely caustic remarks about such work, pointing out very truly, how remote the conditions are from the living state.

While this is all very true, it is not without importance to know what happens to substances in solution inside dead gut. At the present time there is a tendency to observe the effect of various more or less lethal procedures on the power of the small intestine to absorb and to draw conclusions concerning the nature of the absorbing "mechanism".

May it not be that these procedures not infrequently simply cause death of the gut at about the same time as they kill the animal? Is it justifiable to attribute changes in absorbing power to inhibition of any one specific factor when the whole gut, especially the epitheluim is in a state of imminent dissolution?

As far as I know, no such investigation of the "absorptive" powers of dead rat intestine has been done. It was decided, therefore, to determine the absorption rates of glucose and xylose from the intestine

of the dead rat.

## II Methods.

The experimental animal was killed by bleeding to death, by killing with sodium iodoacetate or with ether. It was found afterwards that the method of killing had no effect upon the amount of sugar which subsequently disappeared from the lumen of the gut. Fifteen minutes after death, the abdomen of the rat was opened and the whole small intestine washed out with warm Ringer at 38°C. Excess moisture was removed by blowing air gently through the small gut. A loop of jejunum, 20 cms. in length was measured off and a ligature tied round the caudal end of the loop. 1.5 ccs of blood isotonic glucose (5.4%) or blood isotonic xylose (4.5%) were run into the jejunal loop from a microburette by means of a cannula tied into the cranial end of the loop. The cranial end was then tied off, the abdomen sewn up and the animal left in a warm environment for one hour. At the end of this period, the abdomen was re-opened and the contents of the loop washed into a 25 cc. graduated cylinder and made up to the mark with distilled water. The sugars were determined quantitatively by the method of Hagedorn and Jensen.

## III. Results.

The results obtained from 18 rats are given below in Table XII.

The mean "absorption" rate for glucose, one quarter of an hour after the death of the animal, is 40.34 mgms per hour from a twenty centimetre loop of intestine. Converting this into a percentage,

Table XII

| Rat No. | Hrs. after death | Sugar used | Amt. absorbed per hour. |
|---|---|---|---|
| 1 | 0.25 | Glucose | 37.92 mgms |
| 2 | 0.25 | " | 41.60 mgms |
| 3 | 0.25 | " | 41.52 mgms |
| 15 | 0.25 | " | 33.75 mgms |
| 16 | 0.25 | " | 56.75 mgms |
| 17 | 0.25 | " | 38.25 mgms |
| 18 | 0.25 | " | 24.00 mgms |
| 19 | 0.25 | " | 38.25 mgms |
| 21 | 0.25 | " | 53.25 mgms |
| 22 | 0.25 | " | 39.50 mgms |
| | Mean | | 40-34 mgms |
| | | | 49.8% absorption. |
| 13 | 0.25 | Xylose | 37.75 mgms |
| 14 | 0.25 | " | 37.50 mgms |
| 24 | 0.25 | " | 36.75 mgms |
| 25 | 0.25 | " | 36.25 mgms |
| 26 | 0.25 | " | 26.25 mgms |
| 27 | 0.25 | " | 26.75 mgms |
| | Mean | | 33.6 mgms |
| | | | 50.1% absorption |

49.8% of the glucose disappeared from the lumen of the gut in one hour. Under identical conditions, the mean "absorption" rate for xylose was found to be 33.6 mgms. Expressed as a percentage, 50.1% of the xylose disappears from the lumen of the small intestine.

## IV Discussion.

This is postponed until the end of the next section.

## V. Summary.

The rates of "absorption" of glucose and xylose from the jejunum of a rat killed a quarter of an hour beforehand were determined. In each case, 50% of the sugar is "absorbed".

## SECTION D.

Post-Mortem Changes in the Epithelium of the Small Intestine of the Rat

Post-Mortem Changes in the Epitheluim of the Small Intestine of the Rat.

## I. Introduction

The striated-border epitheluim of the small intestine is extremely delicate and can be injured by procedures not directly applied to the intestine. It was Waymouth Reid who first drew attention to this fact in 1900. Interruption of the circulation to the gut for from 15 to 30 minutes by clamping the mesenteric arteries caused complete desquamation of the intestinal epitheluim. In his own words"the epithelium of the intestine is very loosely attached".

In view of this fact it was decided to make a systematic histological examination of the small intestine of the rat at varying intervals after the death of the animal.

## II Method.

Five rats were killed with coal-gas and the time of death noted. After ¼ of an hour the abdomen of the first rat was opened up and pieces of small intestine placed in picro-dioxan. Similarly, ½ an hour, 1 hour, 2 hours and 4 hours respectively after death pieces of small intestine from the remaining four rats were removed and placed immediately into fixative.

Another group of four rats were killed, this time by bleeding. Pieces of small intestine were removed from each rat at a varying interval after death and fixed immediately in picro-dioxan.

The tissues were subsequently embedded in paraffin. Sections were cut and stained with Erlich's Acid Haematoxylin and Orange G.

## III. Results.

It was evident that the method of killing had no effect upon the histological picture of the small intestine of dead rats.

Histological examination of the intestine in dead rats shows at once that the intestinal epithelium is grossly injured within a very short time.

Fig. 8 shows the normal appearance of the rat's jejunum. The epithelium is absolutely intact and adherent to its basement membrane. It is significant that in order to obtain such a picture it is necessary to remove the gut from a living anaesthetised rat and to place the tissue at once in picro-dioxan.

The appearance of the gut removed from a rat fifteen minutes after death is shown in Fig. 9. Already desquamation is apparent at the tips of the villi.

Fig. 10 shows the condition of the mucous membrane 1 hour after death. Desquamation of the epithelium is more advanced and disorganisation of the villous structure is beginning.

It can be seen in Fig. 11 that gross disorganisation of the villi has already taken place 2 hours after death and is continued until 4 hours after death, the disorganisation being that which one sees in Fig. 12.

## Fig. 8.

Fig. 8 shows the normal appearance of the rats jejunum. The epithelium is absolutely intact and adherent to its basement membrane. But, in order to obtain such a picture it is necessary to remove the gut from a living anaesthetised rat and to place the tissue at once in fixative.

Fig. 9.

Fig. 9 shows the appearance of gut removed from a rat 15 minutes after death. Already desquamation is apparent at the tips of the villi (a).

## Fig. 10.

Fig. 10 shows the condition of the mucous membrane of the gut 1 hour after the death of the rat. Desquamation of the epithelium is more advanced and disorganisation of the villous structure is beginning.

Fig. 11.

Fig. 11 pictures the gross disorganisation of the villi 2 hours after the death of the rat.

Fig. 12.

Fig. 12 shows the gross disorganisation of the whole wall of the small intestine of the rat four hours after death. As the tissues lose their substance they loose their affinity for histological stains. Before fixation, the segments resembled short tubes of opaque cellophane.

## IV. Discussion.

1) Absorption of Glucose and Xylose from the Intestine of the Dead Rat.

It is evident that although the intestinal villi may be denuded of the epithelial cells, surprisingly large amounts of glucose and xylose diffuse out of the lumen of the gut. In addition, the percentage "absorption" of each sugar is the same, that is to say, the ratio $\frac{\text{percentage "absorption" glucose}}{\text{percentage "absorption" xylose}}$ has become $\frac{1}{1}$. In the living, normal rat the ratio is between $\frac{5}{1}$ and $\frac{4}{1}$. The selective absorption of glucose has been abolished on the death of the animal.

Cohnell and Hoeber (1939) found that the selective absorption of glucose from the perfused small intestine of the rat disappeared after half an hour. The disappearance of the selective absorption of glucose coincided with the appearance of considerable amounts of cellular débris within the lumen of the gut. In spite of the work of Auchinachie, MacLeod and Magee (1930) it is well known that the surviving loops of intestine used for in vitro work soon lose their power of absorbing glucose preferentially.

One can say, therefore, that the integrity of the epithelium is essential for the selective absorption of glucose. In view of this fact, any experimental procedure which abolishes the selective absorption of glucose should be subjected to vigorous histological control.

2) Histological Examination of the Epithelium.

Since the same fixative namely picro-dioxan was used to fix the tissues from the living rat and the dead rats it is obvious that neither the fixative nor the subsequent process of paraffin embedding caused

desquamation of the intestine.

Waymouth Reid (1900) found that it was difficult to interrupt the circulation to the gut even for very short intervals without detaching the intestinal epithelial cells from the basement membrane. Five minutes was the maximum time the mucous membrane could be denied its blood-supply without desquamation of the epithelial cells. Similarly, desquamation of the mucous membrane within a very short period after death is shown in Fig. 9. The cause of detachment of the intestinal epithelial cells from the basement membrane is unknown. It may be directly due to cessation of the blood supply to the gut or indirectly to enzymatic action following upon interruption of the blood supply to the intestine.

## V. Summary.

Histological examination of the small intestine of rats removed at varying intervals after death showed that there is progressive desquamation of the epithelial cells and disorganisation of the substance of the villi.

Under such conditions the selective absorption of glucose is abolished, suggesting that the factors responsible for the preferential absorption of glucose reside principally in the epithelial layer.

# SECTION E.

The Condition of the Mucous Membrane in Surviving Isolated Intestine.

I. Introduction.

II. Methods.

III. Results. a) Rabbit.

b) Rat.

IV. Discussion.

V. Summary.

## The Condition of the Mucous Membrane in Surviving Isolated Intestine.

### I. Introduction.

Auchinachie, MacLeod and Magee (1930), in a series of experiments designed to measure the rate of diffusion of glucose and xylose from the intestine allowed diffusion to occur through the walls of isolated portions of intestine kept in a bath of oxygenated saline solution at body temperature. They believed that such preparations retained the absorptive powers of gut in situ since they found that loops of dead gut killed by heat or protoplasmic poisons, allowed more rapid diffusion of solutes from lumen to surrounding Ringer's fluid than did surviving gut. Living segments were said also to exhibit the property of selective absorption. For example, xylose diffused more rapidly through dead segments at body temperature than did glucose, the reverse being the case with surviving isolated segments., At low temperatures in the case of living segments, xylose diffused more rapidly than glucose. The increased rate of diffusion of glucose was less marked between 0°C and 20°C than between 20°C and 40°C. The absorption results were determined over a period of two to three hours.

On the face of it, such an experimental technique is open to grave criticism principally since normal absorption from the gut lumen does not involve passage of the absorbed material through the entire gut wall into the cavity of the peritoneum. Moreover, in view of the results obtained in Section D with segments of intestine removed at varying intervals after the death of the animal, it was thought that it would be useful to examine histologically the condition of the mucous

membrane of segments of isolated surviving intestine and to find if there was any similar desquamation of the epithelial cells. Such an investigation was all the more necessary since the results of MacLeod and his co-workers are widely accepted as having a bearing on the problem of true intestinal absorption.

## II Methods.

A full-grown rabbit was killed by a blow on the head and a loop of small intestine about twenty centimetres long was immediately removed with great care in handling. The loop was suspended in warm oxygenated Ringer at 38°C in a Burn-Dale bath. Fifteen minutes later, a small piece of intestine about two centimetres long was carefully removed from the bath and placed immediately in picro-dioxan. This procedure was repeated thirty-minutes, sixty minutes and one hundred and twenty minutes after the beginning of the experiment. The tissues were embedded in paraffin and subsequently cut and stained in the usual fashion.

Similarly in the case of the rat the condition of the epithelium of segments of small intestine kept for given periods of time in Ringer solution under optimum conditions of temperature and pH was determined.

## III. Results.

### a) Rabbit.

Figs. 13 to 16 show that desquamation of the intestinal epithelium has proceeded rapidly. Within quarter of an hour there are signs that the cells are just beginning to detach themselves from the basement membrane (Fig.13). After half an hour the epithelial cells on the sides of the villi have desquamated leaving a cap of cells still

Fig. 13.

Fig. 13 shows the appearance of an isolated living segment of the small intestine of the rabbit after the segment has been suspended in warm oxygenated Ringer for ¼ an hour. Already desquamation has started (a) Detached epithelial cells can be seen in the gut lumen. (b)

## Fig. 14.

Fig. 14 shows the appearance of an isolated living segment of the small intestine of the rabbit after the segment has been suspended in warm oxygenated Ringer for ½ an hour. Desquamation has progressed further but the desquamation pattern in the rabbit differs from that in the rat. In the rabbit the sides of the villus are denuded first, leaving a cap of epithelial cells still attached to the tip of the villus (a). The lumen of the gut is packed with cellular débris. (b).

## Fig. 15

Fig. 15 shows the appearance of an isolated living segment of the small intestine of a rabbit after the segment has been suspended in warm oxygenated Ringer for an hour. Desquamation of the mucous membrane has progressed even further. The cap of epithelial cells on the tip of the villus is still evident (a) and the lumen of the gut is filled with detached epithelial cells (b).

## Fig. 16.

Fig. 16 shows the appearance of an isolated living segment of the small intestine of a rabbit after the segment has been suspended for 2 hours in warm oxygenated Ringer. Gross disorganisation of the villi is now evident. Even the epithelial cap of cells on the tips of the villi has disintegrated.

attached to each villus. (Fig.14). After two hours even these cells have become detached and the villi are completely disorganised. (Fig.16). The lumen of the loop is packed with desquamated epithelial cells (Figs.13-16). Although the villi of the small intestine have been denuded of epithelial cells the intestinal movements still continue.

b) Rat

Desquamation of the epithelial cells has proceeded been more rapidly in isolated segments of gut kept alive in warm oxygenated Ringer than in segments of intestine which had remained in the dead rat for similar intervlas. Detachment of the dead cells has probably been aided by the additional handling necessary to suspend the loop in a Burn Dale bath. Even after quarter of an hour desquamation of the mucous membrane has already begun (Fig.17). Within half an hour it is well established (Fig.18) and after an hour it is gross. (Figs.19 and 20).

The desquamation pattern of the intestinal epitheluim of the rat is different from that of the rabbit. Detachment of the cells begins at the tip of the villus and proceeds down towards the base.

## IV Discussion

Auchinachie, MacLeod and Magee (1930) define an isolated surviving segment of small intestine as one removed from the animal and placed in warm oxygenated Ringer at 36°C in a Burn-Dale bath. A dead segment of isolated intestine they define as a segment killed by heat or washing with sodium cyanide or sodium fluoride.

According to Auchinachie, MacLeod and Magee the more rapid

## Fig. 17.

Fig. 17 shows the appearance of an isolated living segment of the small intestine of a rat after the segment has been suspended for ¼ an hour in warm oxygenated Ringer. Even after ½ hour desquamation of the epithelial cells is well advanced. Desquamation appears to be more rapid in living isolated segments of rat gut than in dead rat gut after a similar lapse of time.

## Fig. 18.

Fig. 18 shows the appearance of an isolated living segment of the small intestine of a rat after the segment has been suspended for ½ hour in warm oxygenated Ringer. Desquamation of the epithelial cells has progressed rapidly.

Fig. 19.

Fig. 19 shows the appearance of an isolated segment of the small intestine of a rat after a segment has been suspended for an hour in warm oxygenated Ringer. Desquamation of the epithelial cells is now severe.

Fig. 20.

Fig. 20 shows the appearance of an isolated living segment of the small intestine of a rat after a segment has been suspended for two hours in warm oxygenated Ringer. Gross disorganisation of the villi is now evident.

diffusion which occurs through dead as compared with surviving intestinal loops is due to changes of a physico-chemical nature in the walls. They state that this increase in permeability to all diffusible substances is due to coagulation of protein with a consequent change in the colloidal structure of the intestinal walls. However Waymouth Reid in 1900 showed that washing the small intestine with a weak solution of sodium fluoride causes complete desquamation of the mucous membrane. He showed moreover, that interruption of the blood supply to the gut for fifteen to thirty minutes also caused severe desquamation of the intestinal epithelium. In the case of living isolated segments of gut one would expect desquamation of the mucous membrane to become increasingly severe with time as the blood supply to the epithelial cells is completely interrupted. In the case of dead isolated segments the inevitable process of desquamation of the mucous membrane upon interruption of the blood supply has been hastened by washing with fluoride or cyanide. The increase of permeability of dead gut to diffusible substances is probably due to the removal of the epithelial barrier.

If isolated loops of intestine are used to determine the rate of absorption of glucose and of xylose, it must be borne in mind that the absorption rate is being measured across a desquamating membrane. At the beginning of the experiment the intestinal epitheluim is intact, at the end of the experiment little of the epithelium may be left. Before the absorption rate of glucose can be determined it is surely necessary to know whether the diffusion rate across the intact epitheluim is being measured or whether the diffusion rate across the basement membrane is

being measured. In the case of surviving isolated loops of intestine it is difficult to know what exactly is being measured. In comparing the rates of diffusion between living and dead segments of isolated intestine, one is chiefly comparing the rates of diffusion across a desquamating and a desquamated membrane.

## V. Summary.

Segments of living isolated rabbit intestine were suspended in warm oxygenated Ringer at 38°C in a Burn-Dale bath. Small pieces of intestine were removed from the bath fifteen, thirty, sixty and one hundred and twenty minutes after the beginning of the experiment and fixed immediately in picro-dioxan. Tissues were embedded cut and stained. Rat intestine was similarly treated. The epitheluim of these segments kept in vitro for some time was found to desquamate rapidly. The implications of this in connection with in vitro absorption experiments are discussed.

## SECTION F.

The Effect of Local Poisoning with Mono-iodoacetic Acid upon the Intestinal Mucous Membrane of Rats.

I. Introduction.

II. Method.

III. Results.

IV. Discussion.

V. Summary.

## The Effect of Local Poisoning with Mono-iodoacetic Acid upon the Intestinal Mucous Membrane of Rats.

### I. Introduction.

To explain the rapid selective absorption of such sugars as glucose and galactose, Verzár (1936) claimed that these existed in the small intestine a phosphorylation mechanism which promoted the rapid transport of these sugars across the mucous membrane. This phosphorylation hypothesis rested initially on the observation that poisoning rats with mono-iodoacetic acid abolishes such selective absorption. After poisoning with mono-iodoacetic acid glucose is no more rapidly absorbed than xylose. The validity of Verzár's claim that mono-iodoacetic acid is a specific inhibitor of phosphorylation is discussed on page.

Iodo-acetic acid is now known not to be a specific inhibitor of phosphorylation. Nevertheless, it does interrupt the metabolic changes in muscle. It is quite conceivable that iodoacetic acid interferes with processes involved in the preferential absorption of glucose and galactose and absent during the absorption of xylose. However, it is obvious that care should be taken to exclude the other possibility that iodo-acetic acid merely has a gross injurious action upon the gut epitheluim.

Westenbrink (1936), Klinghoffer (1938) and Ochnell and Hoeber (1939) have all hinted that iodoacetic acid acts not so much by inhibition of a chemical process as by disorganisation of the gut epithelium. However as far as I know, they have made no systematic investigation of this point.

In early experiments, Wilbrandt and Laszt (1933) two of Verzár's co-workers placed a solution of blood-isotonic glucose (5.4%), containing 1/5000 mono-iodoacetic inside the lumen of the gut and found that in an hour the percentage of glucose absorbed was reduced from a normal of 72% to 55%. In the case of a blood-isotonic solution of xylose (4.5%), containing 1/5000 iodoacetic acid, the percentage absorption in the poisoned animal was 25.4%. In normal animals, the percentage absorption of isotonic xylose was 21.4%. The ratio $\frac{\text{percentage absorption glucose}}{\text{percentage absorption xylose}}$ in an hour was 2.2/1 in poisoned rats whereas in the normal rats the ratio was 3.4/1. There was, therefore, but slight interference with the selective absorption of glucose by placing 1/5000 iodoacetic acid in the lumen of the gut. Were the iodoacetic acid acting as a specific inhibitor of phosphorylation, or of some other chemical process, one could imagine no better opportunity for the poison to exert its action than when placed in the gut lumen in actual contact with the epithelial cells.

Since nothing was known about the histological state of the intestinal epithelium under such conditions it was decided to make a systematic examination of the mucous membrane of the small intestine at the end of a routine absorption experiment in which there is local poisoning with 1/5000 iodoacetic acid.

## II. Method.

Five rats in all were used.

The rat was injected subcutaneously with a 25% solution of urethane (1.6 mgms per gm. body weight rat). One hour later the abdomen was

opened and the small intestine washed out with a stream of warm Ringer at 38°C. Excess moisture was removed as before by means of a blower. Into a twenty centimetre loop of jejunum was placed 1.5 ccs of an isotonic solution of glucose containing 1/5000 monoiodoacetic acid. The abdomen was sewn up and the animal left in warm surroundings for an hour. At the end of this period the abdomen was opened again and pieces of the loop of jejunum removed and immediately placed in picro-dioxan.

The tissues were embedded in paraffin, sectioned then stained with Erlich's Acid Haematoxylin and Orange G.

III. Result.

Fig.21 shows the condition of the intestinal epithelium after placing an isotonic solution of glucose containing 1/5000 monoiodoacetic acid inside the gut lumen for 1 hour. There is some injury to the mucous membrane, especially at the tips of the villi and a small amount of cellular débris in the gut lumen.

IV. Discussion

It is surprising to find that the percentage absorption of glucose from an isotonic solution of glucose containing 1/5000 mono-iodoacetic acid placed in the gut lumen is so high. If phosphorylation is the active agent in the selective absorption of glucose and galactose and iodoacetic acid inhibits that agent then one would expect to find that local poisoning of the gut would almost completely abolish such preferential absorption. Instead of this, the percentage absorption of glucose is reduced from 74% in the normal animal to 55% in the

Fig. 21.

Fig. 21 is a photomicrograph of the small intestine from a rat anaesthetised with urethane. A solution of 5.4% glucose containing $\frac{1}{5000}$ monoiodoacetic acid was placed in the gut lumen for an hour. There is some injury to the gut epitheluim, especially at the tips of the villi (a).

poisoned animal.

This apparent anomaly removes itself if one considers the histological picture of the mucous membrane in the poisoned animal. In dead animals, in which there was shown to be gross disorganisation of the intestinal epithelium, the selective absorption of glucose was completely abolished. In animals in which the gut is locally poisoned with iodoacetic acid there is only partial desquamation of the intestinal epithelial cells and the selective absorption of glucose is but slightly interfered with.

It is probable that this partial desquamation of the intestinal epithelium would be sufficient to account for the slight depression of the preferential absorption of glucose without postulating inhibition of hypothetical chemical processes inside the epithelial cells.

## V. Summary.

Histological examination was made of the epithelium of the small intestine of rats in which an isotonic solution containing 1/5000 iodoacetic acid had been placed in the small gut for 1 hour.

Partial desquamation of the epithelial cells was found to occur. An attempt is made to correlate this partial desquamation with the slight decrease in absorption rate of glucose which occurs when the gut is locally poisoned.

## SECTION C.

The Effect of Systemic Poisoning with Monoiodoacetic Acid upon the Intestinal Mucous Membrane of Rats.

I. Introduction.

II. Method.

a) Effect of Subcutaneous Injection of Monoiodoacetic Acid into Rats.

b) Subcutaneous Injection of Monoiodoacetic Acid into Rats under Urethane Anaesthesia.

c) Routine Absorption Experiment using Iodoacetic Acid.

III. Results.

a) General Condition of the Animals.

b) Macroscopic Appearance of Alimentary Tract.

c) Microscopic Appearance of Alimentary Tract.

IV Discussion.

V. Summary.

## I. Introduction.

Since the presence of mono-iodoacetic acid in a concentration of 1/5,000 in the actual gut lumen had only a slight action on the preferential absorption of glucose as compared with xylose in subsequent experiments, Willerandt and Laszt (1933) injected mono-iodoacetic acid subcutaneously into rats and found that the selective absorption of glucose was completely abolished. On the other hand the absorption rate of xylose from the small intestine was not affected by iodoacetic acid. Initially it was upon this evidence that Verzár and his co-workers based the theory of phosphorylation to explain the preferential absorption of such sugars as glucose and galactose.

Later workers criticised this theory on the grounds that rats injected with mono-iodoacetic acid were almost moribund. Westenbrink (1936) suggested that the action of monoiodoacetic acid is, in part at least, due to the effect on the circulatory system Klinghoffer(1938) reported that gross pathological changes followed the subcutaneous injection into rats of monoiodoacetic acid in doses varying between 6 and 20 mgms per 100 gms body weight. These changes include pyloric spasm in the gut, adrenal cortical and medullary haemorrhages, haemorrhagic spasm of the gut, haemoglobinuria and pharyngeal oedema. Unfortunately, none of these were consistently present but most of the animals exhibited macroscopic gastro-intestinal lesions. No systematic histological investigation, however, was carried out.

Ochnell and Hoeber (1939) state that rats, injected subcutaneously with iodoacetic acid, exhibit defects in the intestinal epithelium.

Once More, a systematic investigation was lacking. No indication was given of the extent of the damage nor was there any attempt made to follow up the implications of these observations.

In view of these facts it seemed necessary ot investigate fully the action of parenteral administration of monoiodoacetic acid upon the mucous membrane of the small intestine of rats.

## II. Methods.

In all, thirty rats were used. These rats were of two different strains. One group of rats was obtained from the Rowett Research Institute, the other group comprised rats bred in the laboratory.

a) Effect of Subcutaneous Injection of Iodoacetic Acid.

The rats employed weighed approximately 200 gms. They were starved for twenty-four hours before the experiment. Monoiodoacetic acid (B.D.H.) was converted into the sodium salt by the addition of sodium hydroxide until the solution was neutral to phenol red. This solution was then injected subcutaneously into the animals. The dose given varied from 10-16 mgms. per 100 gms. body weight rat. The animals were then kept in warm surroundings for the desired length of time - usually 2½ hours. This was the time rats were exposed to monoiodoacetic in the routine absorption experiments carried out by Wilbrandt and Laszt (1933).

The rats showed varying susceptibility to iodo-acetic acid. With a dose of 16 mgms per 100 gms. rat two were moribund within half an hour. These were killed, the abdomen opened at once and portions of the gut fixed in picro-dioxan. The majority survived a similar dose

for at least two and a half hours, while four were alive five hours after the injection. In all cases, great care was taken to avoid post-mortem changes which, as shown in Section E , set in with great rapidity.

After embedding the tissues in paraffin, sections were cut and subsequently stained with Erlich's Acid Haematoxylin and Orange G.

b) Subcutaneous Injection of Monoiodoacetic Acid into Rats under Urethane Anaesthesia.

Rats were anaesthetised with urethane (1.6 mgms per gm. rat) injected subcutaneously in the form of a 25% solution in distilled water. This was the anaesthetic used by Willbrandt and Laszt in their absorption experiments using iodoacetic acid to abolish the selective absorption of glucose. One hour afterwards, monoiodoacetate was injected subcutaneously, the dose being 10-16 mgms per 100 gms body weight rat. Two and a half hours after the injection of monoiodoacetic acid, the abdomen of the animal was opened, pieces of small intestine removed and fixed in picro-dioxan. In this case, the tissues were removed before the animal was killed. Sections were cut and stained in the usual fashion.

c) Routine Absorption Experiment using Iodoacetic Acid.

The rats were starved for 24 hours and then anaesthetised with urethane (1.6 mgms per gm. rat) injected subcutaneously. Monoiodoacetic acid (10-16 mgms per 100 gms rat) was injected subcutaneously when anaesthesia was obtained. When iodoacetate had been allowed to act for one and a half hours, the abdomen was opened and the small

intestine washed out with a gentle stream of warm Ringer at 38°C. 1.5 ccs of blood-isotonic glucose solution (5.4%) was placed in a loop of jejunum. The animal was sewn up and left in a warm environment for an hour. At the end of this period small pieces of the loop of intestine were removed and fixed in picro-dioxan. Sections were cut and stained. In this way, the histological appearance of the small intestine in iodoacetate - poisoned rats during the absorption of glucose was determined.

## III Results.

### a) General Condition of the Animals.

Often within quarter of an hour of the injection of iodoacetate the behaviour of the animal altered. The hind legs became paralysed very quickly. After half an hour, the animal appeared to be very ill. It soon became prostrate, the body temperature fell and tremors were evident. Respiratory distress and violent convulsions ushered in a state of coma. In a few cases the rats expelled blood-stained faeces.

### b) Macroscopic Appearance of Alimentary Tract.

On opening the abdomen there was a characteristic unpleasant odour. The intestinal tract appeared to be particularly affected by iodoacetic acid. It was evident that there was spasm of the pyloric sphincter and on opening the stomach, it was found that there was present bile-stained fluid in some quantity. The small intestine was congested and here and there were definite patches of dark plum-coloured gut suggesting complete circulatory stasis or infarction. Not infrequently

there was blood in the gut lumen. The large intestine appeared to be unaffected.

c) Microscopic Appearance of the Alimentary Tract.

Microscopically, there was widespread injury to the epithelium of the small intestine. The lesions were by no means uniform, some regions being only slightly damaged others showing the grossest possible disorganisation. Fig.22 is a typical section from the intestine of an anaesthetised rat injected with 16 mgms monoiodoacetic acid per 100 gms rat. The villi are denuded of the striated border epithelium and even the cores of the villi shows signs of disorganisation.

In the lumen of the small gut there were large amounts of cellular débris. The capillaries of the small intestine were obviously dilated. The colon and caecum were free from such lesions.

All rats injected as in IIa, IIb and IIc were found to have lesions of varying degrees of severity in the small intestine.

## IV Discussion.

In view of these results it is surprising to find Verzár state that it was decided to inject the monoiodoacetic acid subcutaneously rather than place it along with the sugar solution inside the gut lumen "in order to avoid direct damage to the intestinal epithelium". (Verzár and McDougall, 1936).

Comparison of Figs. 21 and 22 shows that general poisoning with iodoacetic acid causes more extensive disorganisation of the intestinal mucous membrane of the small intestine than local poisoning of the gut with iodoacetic acid. This suggests that the injurious action of

Fig. 22.

Fig. 22 shows the marked injury to the gut epithelium in a rat which had received sodium iodoacetate subcutaneously, 3½ hours previously c.f. Fig. 21.

iodoacetic acid upon the intestinal epithelium is indirect rather than direct. If the action of iodoacetic acid upon the epithelium had been direct, the results obtained ought to have been the reverse. Since this injurious action of iodoacetic acid upon the gut mucous membrane is a secondary action, one must determine the system or structures primarily affected by the poison.

In dead rats, where the circulation to the gut has ceased, the histological picture of the epithelium of the small intestine is identical with that of rats injected subcutaneously with iodoacetic acid. In iodoacetate - poisoned rats the circulatory system is obviously upset, there being cyanotic congestion of the small intestine and dilation of the capillaries. Furthermore, Waymouth Reid (1800) found that interruption of the circulation to the small intestine even for short intervals caused extensive desquamation of the epithelial cells.

It is possible that iodoacetic acid injected subcutaneously acts directly upon the circulatory system resulting in an inadequate blood supply to the epithelial cells of the mucous membrane. As a result these cells die and desquamate. This explanation would account for the less injurious action of monoiodoacetic acid when in direct contact with the mucous membrane.

In dead animals, although the epithelium of the small intestine has desquamated, "absorption", if one can call it such, can still occur. Similarly, in rats poisoned with iodoacetate, sugars can still be "absorbed" since the underlying tissues seem to present no barrier to the diffusion of substances from the gut lumen. In the poisoned

rats and in the dead rats the ratio $\frac{\text{percentage absorption glucose}}{\text{percentage absorption xylose}}$ is $\frac{1}{1}$. In both poisoned rats and dead rats extensive desquamation of the mucous membrane of the small intestine occurs.

It is clear that the abolition of the selective absorption of glucose in iodoacetate poisoned rats is due to the desquamation of the epithelial cells of the small intestine resulting from a circulatory upset caused by the iodoacetate. It is not profitable to discuss whether phosphorylation is inhibited within the intestine epithelial cells when few epithelial cells remain in iodoacetate-poisoned rats. Phosphorylation of certain sugars may occur in the mucous membrane of the small intestine but iodoacetic acid certainly cannot be employed to prove the existence of this chemical process. Any procedure, in fact, designed to investigate the nature of selective absorption must not damage the intestinal mucosa. With regard to the use of iodoacetic acid, this ideal has not been attained.

## V. Summary.

In contrast to local poisoning of the small intestine of rats with mono-iodoacetic acid, subcutaneous injection of this poison causes gross disorganisation of the mucous membrane and circulatory defects in the blood supply to the small intestine. It is suggested that iodoacetic acid has a direct action upon the circulatory system causing stasis in the small intestine in particular. This in turn results in desquamation of the epithelial cells. This desquamation is sufficient to account for the abolition of the preferential absorption of glucose without postulating the inhibition of a chemical process inside the epithelial cells.

particularly when few epithelial cells remain attached to the basement membrane.

# SECTION H.

## The Effect of Other Toxic Agents upon the Epitheluim of the Small Intestine

I. Introduction.

II. Methods.

a) Sodium Fluoride.

b) Sodium Cyanide.

III. Results.

a) Sodium Fluoride.

b) Sodium Cyanide.

IV. Discussion.

V. Summary.

## I. Introduction.

Sodium Fluoride was known to inhibit phosphorylation processes in muscle metabolism at a later stage than iodoacetic acid (Lohmann, 1931). Verzár and his co-workers tried the effect of injecting sodium fluoride intravenously into rats then determining the rates of absorption of glucose and xylose. Rather surprisingly, sodium fluoride did not reduce the amount of glucose and xylose absorbed. Moreover, the ratio $\frac{\text{percentage glucose absorbed}}{\text{percentage xylose absorbed}}$ in one hour remained normal. The action of sodium fluoride, therefore, differs from that of iodoacetate even although both substances inhibit phosphorylation processes in muscle.

Sodium cyanide was also used by Verzár as a general poison of respiratory enzymes. He injected the cyanide subcutaneously. Results were erratic, there being interference in some case with the absorption of both glucose and xylose. Nothing is known of the histological appearance of the gut under such conditions.

## II. Methods.

a) Sodium Fluoride.

The rats were starved for 24 hours then lightly anaesthetised with ether. The femoral vein was exposed and a solution of sodium fluoride - 0.3 mgms per $10^0$ gms rat - injected into it. The wound was sewn up and the animal allowed to recover from the anaesthetic. After the sodium fluoride had been allowed to act for an hour, the animal was again lightly anaesthetised and pieces of small intestine removed and fixed in picro-dioman. After embedding in paraffin sections were cut and stained.

Injection of sodium fluoride caused acute respiratory distress and the animal was prostrate within twenty minutes of the injection. The survival time was between one and two hours.

b) Sodium Cyanide.

Sodium cyanide was injected subcutaneously into rats previously starved for 24 hours. The dose was 0.8 mgms per 100 gm. rat. One hour after the injection of cyanide the animal was anaesthetised with ether and pieces of small intestine removed and fixed in picro-dioxan. After embedding in paraffin sections were cut and stained.

Injection of sodium cyanide caused violent convulsions within a few minutes of the injection. The animal soon became prostrate and looked seriously ill. Survival time was one day.

## III. Results.

a) Sodium Fluoride.

Macroscopically, the dose of sodium fluoride had no injurious action upon the small intestine. This is somewhat surprising considering the naturally grave symptoms of sodium fluoride poisoning. Fig 23 shows that, microscopically, too, there was no damage to the intestinal epithelial cells. The striated border epithelium is intact and firmly attached to the basement membrane.

b) Sodium Cyanide.

In the case of rats injected with sodium cyanide, there were definite areas of damage in the small intestine. Macroscopically patches of plum-coloured gut could be seen suggesting circulatory stasis. Microscopically, the damage to the intestinal epithelium is shown in Fig.24. The desquamation of the epithelial cells is not so complete as in the iodoacetate-

Fig. 23.

Fig. 23 shows that fluoride injected intravenously in the dose used by Wilbrandt and Laszt has no injurious action on the intestinal epithelium of the rat. The epithelial cells are still attached to the basement membrane.

Fig. 24.

Fig. 24 shows the areas of definite injury in the small intestine of the rat after subcutaneous injection with sodium cyanide (a). Side by side with the desquamated areas are regions where the intestinal epithelium is intact (b).

poisoned animal yet there are areas in which the damage is serious. Similarly, there are patches of small intestine where the gut appears normal and the epithelium is intact.

## IV Discussion.

Initially, Verzár based his phosphorylation theory of carbohydrate absorption on the observation that iodoacetic acid, known to inhibit the phosphorylation processes in muscle, decreased the rate of absorption of glucose and galactose but not xylose. Verzár was, therefore, surprised to find that sodium fluoride, also known to inhibit phosphorylation processes in muscle, did not affect the absorption rate of any of the monosaccharides. Yet these seemingly anomalous results can be explained if the histological pictures of the small intestine of iodoacetate-poisoned and of fluoride-poisoned rats are examined and compared. In the former, desquamation of the intestinal epithelium is almost complete, in the latter, the epithelium is undamaged. In iodoacetate-poisoned rats there is desquamation of the intestinal epithelium followed by a decrease in the rate of absorption of glucose; in the fluoride-poisoned rats there is no desquamation of the epithelial cells and the absorption of glucose is normal. In both cases, the phosphorylation processes said to be necessary for the selective absorption of glucose are presumably inhibited - if such processes exist - yet only where severe desquamation of the intestinal epithelium occurs is there any decrease in the rate of absorption of glucose. Even although phosphorylation of glucose is inhibited by chemical means the rate of absorption of glucose is unaltered as long as the integrity of the

the epithelium is preserved.

In the case of sodium cyanide the distribution of the areas of injured intestinal epithelium follows no definite pattern. This explains the erratic results obtained by Verzár. If one loop of intestine showed no injury to the epithelial cells, the absorption rate of glucose would be normal. In a loop where there were several patches of desquamated villi, the rates of absorption would be appreciably lowered. Moreover, the general condition of the animals is even worse than that of the iodoacetate-poisoned rats.

## V. Summary.

Sodium fluoride - 0.3 mgms/100 gms rat - was injected intravenously into rats previously starved for 24 hours. To desquamation of the intestinal epithelium occurred at the end of one hour. Wilbrandt, and Laszt found no decrease in rate of absorption of glucose in fluoride-poisoned rats. These results are in keeping with the histological findings.

Sodium cyanide - 0.8 mgms per 100 gms rat - was injected subcutaneously into rats. Patchy congestion of the gut resulted with areas of desquamation of intestinal epithelial cells. The histological evidence was in keeping with the erratic results on the absorption of glucose and xylose obtained by Verzár's co-workers.

# SECTION I.

## The Effect of Adrenalectomy upon the Epithelium of the Small Intestine.

I. Introduction.

II. Method.

III. Results.

IV. Discussion.

V. Summary.

## I. Introduction.

Since adrenalectomy was thought to have some slight influence on carbohydrate synthesis in the tissues generally, Wilbrandt and Lengyel (1933) decided to test whether adrenalectomy influenced the processes involved in the selective absorption of glucose from the intestine of the rat.

They found that the mean absorption rate for glucose in the adrenalectomised rat was 41.0 (± 6.3) per cent of the administered glucose in an hour. The normal mean value for rats was 71.6 (±-9.2) per cent. The values for rats showed good agreement with one another but those for adrenalectomised rats varied from 17.9 per cent to 56.1 per cent. The mean absorption rate of xylose was 18.2 (±3.8) per cent in the adrenalectomised rat and 18.0 (± 3.5) per cent for normal rats. Injection of eucortone, probably a mixture of adrenocortical hormones, restored to normal rate the absorption of glucose in adrenalectomised rats. From these experiments, Wilbrandt and Lengyel concluded that the adrenal cortex had a specific rôle in the selective absorption of glucose.

In view of the results found after histological examination of the small intestine of iodoacetate-poisoned rats it was decided to make a systematic histological examination of the small intestine of adrenalectomised rats.

## II. Method.

Rats weighing 150-200 gms. body weight were used. Adrenalectomy was carried out by the lumbar route from a dorsal midline incision made through the skin at the level of the kidneys. The gland was freed from the kidney so that the only attachments were through the pedicle of the adrenal. The pedicle was clamped with a pair of artery forceps and the adrenal cut off completely. The forceps were left in position for one or two minutes to prevent haemorrhage. The other adrenal gland was removed in a similar fashion and the wound sewn up "Nembutal" combined with ether were used as anaesthetics. After the operation the animals were kept in clean dry cages at a temperature of 28°C. At varying times after the adrenals had been removed the animals were anaesthetised with ether, pieces of small intestine removed and fixed in picro-dioxan. The tissues were embedded in paraffin, cut and stained with Erlich's Acid Haematorylin and Orange G.

## III. Results.

Sections of small intestine removed from rats three, six and fourteen days after the operation and after exposure to cold for from 12 to 24 hours were normal. The intestinal epithelial cells were intact and attached to the basement membrane.

After adrenalectomy, adult rats live many days, even weeks or months. They ultimately die in an asthenic condition, refuse food and death is often ushered in by circulatory collapse. Any additional strain, such as anaesthetisation, operation even exposure to cold may precipitate an acute "adrenal deficiency" in adrenalectomised rats. In young rats

exposure to cold is deliberately used to create such deficiency and to allow assay of cortical hormone (Vogt, 1943). I kept my adrenalectomised rats in a refrigerator in an attempt to precipitate an adrenal crisis. In these relatively mature rats, exposure to cold was not very effective. The condition of the rats deteriorated but there was no collapse.

## IV. Discussion.

Without exception, the evidence advanced since the publication of Verzár's original theory of the function of the adrenals in glucose absorption has failed to substantiate his claims. The absorption rate of glucose is lessened in the adrenalectomised rat but not to the same extent as in the iodoacetate poisoned rat. In the iodoacetate poisoned rat the ratio $\frac{\text{percentage absorption glucose}}{\text{percentage absorption xylose}}$ was 1.1/1. In the adrenalectomised rat the ratio was 2.3/1 indicating that the selective absorption of glucose is by no means completely abolished.

On the other hand, an adrenalectomised rat maintained on sodium salts, absorbs glucose at the normal rate (Denel, Hallmann, Murray and Samuels, 1937; Althausen, Anderson and Stockholm, 1939; Clark and Mackay, 1942). One explanation offered is that maintenance of a normal absorption rate depends directly upon the maintenance of a normal appetite and food intake. This is definitely one result of salt intake. Normal rats fasted for 48 hours showed a decreased rate of absorption of the same order as that found in adrenalectomised rats (Cori, C.F and Cori, G.T., 1937). Marazzi (1940) has also reported that fasted normal rats, sham operated and unilaterally adrenalectomised rats show a decreased rate of absorption of glucose comparable with

that found in adrenalectomised rats. Adrenalectomised rats definitely lose appetite. Moreover, adrenalectomised rats do not stand operation well. It is quite conceivable that Verzár's adrenalectomised rats, as a result of urethane anaesthesia and abdominal operation, were showing circulatory collapse to a degree sufficient to explain the observed decrease in absorption. In view of my failure to find any defect in the integrity of the intestinal mucous membrane, one or other of these explanations may be feasible.

The evidence available at the present moment seems to indicate that the adrenal cortex has little or no influence on phosphorylation processes in the gut concerned with the selective absorption of certain hexoses, if such phosphorylation processes exist. Moreover, Lundsgaard and Wilson (1934) were unable to show any action of adrenalectomy on hexose phosphate formation in muscle.

## V. Summary.

A histological examination of the small intestine of adrenalectomised rats showed that no desquamation of the epithelial cells had occurred. The defect in glucose absorption in adrenalectomised rats must be due to other factors, possibly anorexia and decrease in food intake or circulatory collapse.

## GENERAL DISCUSSION.

The most obvious criticism which can be directed against Verzár's phosphorylation theory is the fact that he worked with one species of animal only, namely the rat. If glucose is selectively absorbed as the result of the existence of a phosphorylating mechanism within the intestinal mucosa, absence of such selective absorption in a given species may be taken as evidence of the absence of a phosphorylating mechanism.

For example, Davidson and Gerry (1940) found that within the caudal half of the ileum of the cat the rates of absorption of glucose and xylose were practically equal. If their results are expressed in terms of percentage absorption in order that these results may be comparable with those of Wilbrandt and Laszt 35.8 per cent glucose was absorbed and 40.5 per cent xylose was absorbed during a period of ninety minutes. The ratio $\frac{\text{percentage glucose absorbed}}{\text{percentage xylose absorbed}}$ was 0.89: 1 in comparison with the higher ratio of 1.1: 1 which Wilbrandt and Laszt (1933) obtained in iodoacetate poisoned rats. In such rats they considered that the phosphorylating mechanism responsible for the selective absorption to have been completely inhibited and the glucose consequently absorbed at its diffusion rate. In the distal ileum of the cat, therefore, phosphorylation of glucose in the mucous membrane during absorption of the sugar does not seem to exist. This is in keeping with Lundsgaard's finding that organic phosphate does not accumulate in the intestinal mucosa of cats during glucose absorption while it does do so in rats.

Roy and Sen (1943) found that in the guinea-pig the rate of absorption of glucose, expressed in terms of percentages, was 56.8 per cent while the rate of absorption of xylose was 47.8 per cent. The ratio $\frac{\text{percentage absorption glucose}}{\text{percentage absorption xylose}}$ was 1.18: 1 in the guinea-pig indicating that the phosphorylation mechanism is probably absent in the small intestine of the guinea-pig.

It may be argued that phosphorylation is more active in the cranial regions of the small intestine than in the caudal regions. But it has been shown in Section A that the greater absorbing power of the cranial regions of the small intestine can be explained on the purely physical basis of greater mucosal surface area in duodenum and jejunum. Ray and Sen (1943) found that, in the guinea-pig, the rate of absorption of xylose was uniform along the gut while glucose was absorbed 26 per cent faster at the ileac end. Eiler, Stockholm and Althausen (1940) showed that during the absorption of glucose at an accelerated rate in a thyroxinised rat there is no significant difference in the concentrations of the several fractions of the acid soluble phosphate in the mucosa of the upper quarter and lower three quarters of the intestine of the rat. This also supports the view that the activity of the phosphorylating mechanism is no greater in the cranial regions of the small intestine than the caudal regions.

Verzár's method of expressing the rates of absorption of isotonic sugar solutions if not disingenuous is certainly not altogether desirable. He expresses the amount of sugar absorbed as a percentage of the total amount of sugar initially placed inside the loop of intestine.

Wilbrandt and Laszt (1933) express the mean absorption rate of glucose from the intestine of the normal rat as 73.3 per cent in one hour and the mean absorption rate of xylose as 21.8 per cent in one hour. Iodoacetate poisoned rate absorbed glucose at the mean rate of 24.3 per cent in one hour and xylose at the mean rate of 22.8 per cent in one hour. This suggests that iodoacetate poisoning completely obliterates the mechanism for the selective absorption of glucose. Since 3 c.c.s of blood-istonic glucose or galactose were placed in a loop of intestine and 3 c.c.s of blood-istonic xylose were placed in another loop the ratio $\frac{\text{amount glucose absorbed}}{\text{amount xylose absorbed}}$ may give a different picture, Table XIII.

TABLE XIII.

| | Normal Initial Amt. Mgms. | Amt. absorbed in 1 hr. mgms. | Poisoned Initial amt. mgms | Amt. absorbed in 1 hr. mgms. |
|---|---|---|---|---|
| Galactose | 162 | 132 = 81.8% | 162 | 64.3 = 39.7% |
| Glucose | 162 | 118 = 73.3% | 162 | 39.4 = 24.3% |
| Xylose | 135 | 28 = 21.8% | 135 | 30.8 = 22.8% |
| $\frac{\text{Percentage Glucose absorbed}}{\text{Percentage Xylose absorbed}}$ | | $\frac{3.4}{1}$ | | $\frac{1.1}{1}$ |
| $\frac{\text{Mgms. Glucose absorbed}}{\text{Mgms. Xylose absorbed}}$ | | $\frac{4.0}{1}$ | | $\frac{1.3}{1}$ |
| $\frac{\text{Percentage Galactose absorbed}}{\text{Percentage Xylose absorbed}}$ | | $\frac{3.7}{1}$ | | $\frac{1.3}{1}$ |
| $\frac{\text{Mgms. Galactose absorbed}}{\text{Mgms. Xylose absorbed}}$ | | $\frac{4.6}{1}$ | | $\frac{2.9}{1}$ |

Expressing glucose and xylose rates of absorption in terms of percentage absorption, the ratio $\frac{\text{glucose absorbed}}{\text{xylose absorbed}}$ was $\frac{3.4}{1}$ in the normal and $\frac{1.1}{1}$ in the poisoned rat. Using absolute values the ratio $\frac{\text{glucose absorbed}}{\text{xylose absorbed}}$ fails to reach the ratio

1/1. This is even more striking in the case of galactose. Administration of iodoacetate, therefore, does not reduce completely the absorption rate of glucose and galactose to the absorption rate of xylose.

The phosphorylation of glucose by mucosal extracts in vitor has been criticised by Westenbrink (1936). Westenbrink quotes Kay that glycerine is much more easily phosphorylated than glucose yet Wilbrandt and Laszt (1933) used a glycerine extract of gut mucous membrane to show the in vitro phosphorylation of certain monosaccharides. According to VerzÁr and Laszt (1934) phosphorylation of glycerol in the intestinal epithelial cells is a pre-requisite for the resynthesis of neutral fat within these cells during fat absorption. Actually, the whole problem of fat hydrolysis and fat absorption is by no means settled and the matter is being actively studies by Frazer (1940).

In an attempt to test the validity of the hypothesis that phosphorylation is concerned with the selective absorption of certain hexoses, Laszt, and Süllmann (1935) studies the effect of the absorption of sodium chloride, galactose, glucose and glycerol on the concentration of the several fractions of the acid soluble phosphate in the intestinal mucosa. Their results showed that the concentration of the organic acid soluble phosphate accompanying the absorption of hexoses and glycerol exceeded the concentration that was observed during the absorption of sodium chloride. However, since the order of increases that were deserved did not correspond with the order of velocity of the absorption of the respective hexoses, it was concluded that no statement could be made concerning the specificity of these changes.

Eiler, Stockholm and Althausen (1940) compared the concentrations of the several fractions of the acid soluble phosphate in the intestinal mucosa during the absorption of glucose in the normal, thyroxinised and thyroidectomised female rat. They found that variations in the velocity of the absorption of glucose, as affected by the thyroid gland, did not cause any significant changes in the concentrations of the several fractions of the acid soluble phosphate in the mucosa of the intestine of the rat. Although absorption of glucose was accompanied by a slight increase in the percentage of the acid soluble esters over that observed during the absorption of sodium chloride, changes in the rate of glucose absorption, as influenced by the thyroid gland, had little effect on the concentration of these esters. Lack of correspondence between the concentration of acid soluble phosphate esters and the velocity of the absorption of glucose neither supports nor invalidates the hypothesis that phosphorylation is concerned with the selective absorption of glucose. It must be assumed that the concentration of these esters depends on the rate of formation and the rate of disappearance. Accordingly, a difference in the rate of turnover of the acid soluble phosphates need not be accompanied by a change in the concentration.

Northup and Liere (1941) studies the effect of anoxia down to and including 54 mms. Hg partial pressure of oxygen on the rate of absorption of glucose from the intestine of the dog. They found that, under such conditions, the absorption of glucose was not significantly altered. Anoxia at 53 mm. Hg, but not higher partial pressures, significantly depressed the absorption of glycine. Colowick, Welsh and Cori (1940)

found that, in kidney extracts, oridation of a dicarboxylic acid is necessary for the phosphorylation of glucose. Colowick, Kalckar & Cori (1941) further state that phosphorylation precedes this oxidation the latter process being necessary for the continuance of the reaction. If absorption of glucose by the intestinal mucosa is dependent on phosphorylation it is rather surprising, in view of the severe anoxia to which the dogs were subjected, that oxygen lack retarded the absorption of glycine but not of glucose

The main weight of criticism against the phosphorylation theory of the selective absorption of glucose has been directed against the use of iodoacetic acid as a means of proving the existence of a phosphorylating mechanism in the intestinal mucosa. Verzár and his co-workers believed iodoacetic acid inhibited phosphorylation indirectly. If it is assumed that the energy required for phosphorylation is derived from the oxido-reduction, inhibition of the latter process will obviously produce an indirect inhibition of the phosphorylation.

Wertheimer considered that impairment of the active absorption of sugar by iod acetic acid was more than a disturbance of the phosphorylation processes and might be indicative of more severe damage to cellular function. According to Luundsgaard and Wertheimer absorption of amino acids is affected by iodoacetic acid although phosphorylation is supposed to play no part in the metabolic reactions of amino acids. Impairment by iodoacetic acid of the following physiological processes has been observed in frogs: the secretion of phenol red and the reabsorption of chloride in the kidney (Ferrari and Höber, 1933; Beck

and Chambers, 1935): the shift of chloride against the concentration gradient by the surviving skin (Huf, 1936); the accumulation and secretion of dyestuffs by the liver (Ferrari and Hober). All these processes are not correlated with phosphorylation but are dependent on the normal energy supply (Koll- Schroed, 1935; Huf, 1936) since after having been abolished by monoiodoacetic acid, they reappear after the addition of lactic acid and pyruvic acid in the presence of oxygen. It is noteworthy that, according to Beck and Chambers, even degenerative processes which would appear under the microscope in the tubular epithelia of the chicken kidney after inactivation by iodoacetic acid could be avoided in the presence of the above named acids.

It has been shown in Section C that injection of iodoacetate into rats causes severe desquamation of the intestinal epithelial cells. Under such conditions inhibition of phosphorylation within the intestinal mucosa is impossible since there is no mucosa left. Histological examination of the gut of iodoacetate poisoned rats shows that the condition of the epithelium is comparable with that of a rat which has been dead for two hours. It is probable that in both cases interruption of the circulation to the gut is the factor responsible for the denudation of the villi.

Laszt (1939) showed that the inhibiting effect of iodoacetate on glucose absorption in rats is suppressed after sodium chloride is injected subcutaneously. The general toxic effect of iodoacetate was said to be counteracted in a similar way. Clark and Barnes (1940) also demonstrated the life maintaining effect of sodium chloride and the lack of effect of cortin.

Iodoacetic acid cannot inhibit phosphorylation, therefore, by its action upon the adrenals. Klinghoffer, moreover, found that in cases of iodoacetate poisoned rats lesions of the adrenal cortex were not consistently present. If iodoacetate abolished the selective absorption of glucose by inhibition of enzymes connected indirectly with the phosphorylating mechanism it is difficult to see how sodium chloride could counteract the inhibiting influence of iodoacetic acid. If, however, iodoacetic acid acted primarily upon the circulatory system, restoration of the selective absorption of glucose by administration of saline is possible. If the circulation to the gut is maintained by the administration of saline, desquamation of the epithelial cells will be avoided and normal absorption of glucose may occur. It is possible that the defect in the absorption of glucose in the iodoacetate-poisoned rat and in the adrenalectomised rat may be due in the former case to failure of the circulation to the small intestine and in the second case failure of the circulation to the small intestine, loss of the balance of electrolytes and anorexia. In both cases administration of sodium chloride restores the selective absorption of glucose.

Danielli (1943) also inclines to the view that poisons inhibiting phosphorylation may prevent absorption not because the substance to be absorbed is phosphorylated but because the metabolism of phosphorylated sugars which might supply the source of energy for some other process concerned in absorption is inhibited. It is surprising however, to find the selective absorption of glucose is not abolished by sodium fluoride, which is known to be a powerful inhibitor of phosphorylation

in muscle. If the energy for the selective absorption of glucose was derived from the metabolism of phosphorylated sugars it would reasonable to expect sodium fluoride to abolish the selective absorption of glucose.

The evidence in favour of the phosphorylation theory involving the use of phosphorylase inhibitors is at the present moment unsatisfactory For example, iodoacetic acid abolishes the selective absorption of glucose but desquamates the intestinal epithelial cells in which ibhibition of the phosphorylating mechanism ought to have taken place. Sodium fluoride another phosphorylase inhibitor, does not abolish the selective absorption of glucose and preserves the integrity of the mucous membrane. Nor is the evidence of phosphorylation of certain sugars in vitro and in vivo so strong that one can accept the phosphorylation hypothesis solely on this evidence. It may be that the stereochemical structure of the sugar molecules will offer some clue to the solution of the problem of absorption of the carbohydrates.

# GENERAL

# SUMMARY

1. A brief historical introduction is given. It deals with the various theories concerning intestinal absorption, beginning about 1800 with the work of Rudolphi.
2. The surface area of the small intestine of the rat and of the cat was measured. Both in the rat and in the cat the mucosal area per unit gut length is much greater in the jejunum than in the ileum.
3. The ratio $\frac{\text{mucosal area}}{\text{serosal area}}$ is greater in the jejunum than in the ileum in both species.
4. The total area of the mucous membrane in the entire small intestine is shown to bear a similar relation to body weight in the cat as in the rat.
5. Using Verzár's technique, the absorption rates of glucose, galactose fructose and of xylose were found in the jejunum and ileum of a large series of rats. The relative rates of absorption were of the same order as those described by Cori and Verzár.
6. In terms of unit mucosal area (see 2 above), glucose, galactose and fructose are absorbed more rapidly in the ileum than in the jejunum of rats.
7. Xylose is more rapidly absorbed per unit mucosal area in the jejunum than in the ileum of rats.
8. Glucose and xylose disappeared from the small intestine of the dead rat rapidly but the rates were equal i.e. the power of selective absorption had disappeared.
9. Progressive desquamation of the epithelial cells of the small intestine of the rat was shown to take place when the animal was killed.

10. The severity of the desquamation varied directly with the time interval after death. This was correlated with the results found in 8.
11. The epithelium desquamated rapidly from loops of intestine of rat and of rabbit when the loops were suspended in war oxygenated Ringer for varying periods of time. In vitro observations therefore are of little value in work on absorption from the gut.
12. The epithelial cells of the small intestine desquamated very slightly when an isotonic solution of glucose containing $\frac{1}{5000}$ iodoacetic acid was placed in the small intestine of rats for an hour.
13. This partial desquamation of the epithelial cells is correlated with the slight decrease in the absorption rate of glucose when the gut is locally poisoned with the addition of $\frac{1}{5000}$ iodoacetic acid to the gut contents.
14. Subcutaneous injection of iodoacetic acid into rats caused severe desquamation of the intestinal epithelium probably as a result of defects in the blood supply to the small intestine.
15. The hypothesis is advanced that this desquamation is sufficient to account for the abolition of selective absorption of glucose in rats injected subcutaneously with monoiodoacetic acid.
16. No desquamation of the epithelial cells of the intestine was found to occur in rats when sodium fluoride was injected intravenously.
17. This is in keeping with the findings of Wilbrandt and Laszt who found no decrease in the rate of absorption of glucose in fluoride poisoned rats although fluoride inhibits phosphorylation in muscle.

18. Sodium cyanide was injected subcutaneously into rats and patchy desquamation of the mucous membrane of the small intestine resulted.

19. The histological findings are in keeping with the erratic results obtained by Verzár and his co-workers on the absorption rates of glucose and xylose after cyanide poisoning.

20. No desquamation of the intestinal epithelial cells occurs in adrenalectomised rats.

21. The present position of the phosphorylation theory is discussed in the light of the findings presented in this thesis.

22. An examination of the logical basis of the phosphorylation hypothesis along with the observations reported in this thesis, throws grave doubts on the whole phosphorylation theory and, moreover, offer an alternative explanation of many of the observations made by other workers and myself.

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